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SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

This issue in Two Parts
PART I—FEBRUARY JOURNAL, SCIENTIFIC EDITION

CONTENTS OF THIS ISSUE

Alpha, Omega-Diphenyl and *Alpha, Omega*-Cyclohexylphenyl Derivatives of Amino Alkanes and Hydroxy Amino Alkanes 65
Walter F. Charnicki and *John B. Data*

The Tryptophane-Nicotinic Acid Relationships and Alkaloids Biosynthesis in Certain Solanaceae 70
M. C. Andries, J. W. Fairbairn, and *H. W. Youngken, Jr.*

A New Approach to the Assay of Alkaloidal Crude Drugs I. Cinchona and Nux Vomica 74
Einar Brochmann-Hanssen

Composition of Gum Turpentines of Pines XXV. A Report on two White Pines: *Pinus koraiensis* from Korea and *P. peuce* from Macedonia 77
P. M. Iloff, Jr., and *N. T. Mirov*

Bacterial Resistance Studies Involving Several Drugs Useful in the Treatment of Urinary Tract Disease 82
Lamar Kerley and *Cecil P. Headlee*

The Use of Hydrogenated Cottonseed Oil and Hexadienol as Suppository Bases 86
Charles W. Hartman and *Joseph P. LaRocca*

A Preliminary Study of the Pharmacology of 11-Desmethoxyreserpine (Raunormine®) an Alkaloid from *Rauwolfia canescens* II 89
Elias W. Packman, Donald D. Abbott, and *Jos. W. E. Harrisson*

Antibacterial Agents in *Pseudomonas aeruginosa* Contaminated Ophthalmic Solutions 93
S. Riegelman, D. G. Vaughan, Jr., and *Masao Okumoto*

A Comparison of Methods of Assay of Digitoxin Tablets 98
W. A. Mannell, A. Lavallee, Rita A. Carioto, and *M. G. Allmark*

Modern Ointment Base Technology I. Properties of Hydrocarbon Gels 101
Margaret N. Mutimer, Charles Riffkin, John A. Hill, and *Gilman N. Cyr*

A Study of Some Factors Influencing the Stability of Pharmaceutical Emulsions I. The Effect of Two-Stage High Pressure Homogenization 105
John D. Mullins and *Charles H. Becker*

A Study of Some Factors Influencing the Stability of Pharmaceutical Emulsions II. The Effect of Wax and Specific Gravity Adjustment 110
John D. Mullins and *Charles H. Becker*

The "Roche" Friabilator 114
E. G. E. Shafer, E. G. Wollish, and *Carl E. Engel*

Effect of Hyperthermic Shock on "Free and Bound" Potassium Levels 116
Charles A. Leonard and *Joseph W. E. Harrisson*

The Measurement of Consistency as a Rheological Property of Ointments 121
Ruth N. Havemeyer

A Note on Zein as a Film-Type Coating for Medicinal Tablets 125
Edward P. Winters and *Dwight L. Deardorff*

Book Notices 126

New and Nonofficial Remedies ..... IV, VI

Advertising ..... Cover pages 2, 3, 4, and I, II, III, V, VI, VIII

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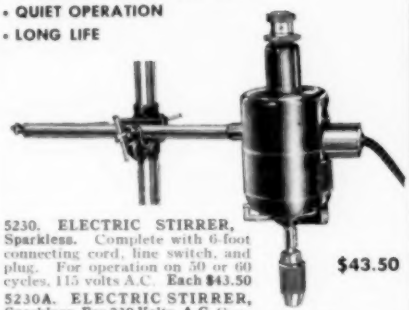

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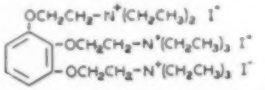
GALLAMINE TRIETHIODIDE.—Flaxedil Triethiodide (Lederle).—$C_{30}H_{60}I_3N_3O_3$.—M.W. 891.56.—[*p* - Phenenyltris(oxyethylene)]tris[triethylammonium iodide].—Tri(diethylaminoethoxy)benzene triethyliodide.—The structural formula of gallamine triethiodide may be represented as follows:



Actions and Uses.—Gallamine triethiodide, a synthetic substituted quaternary amine compound similar in action to curare, is useful to relax skeletal muscle for the same purposes and with the same precautions as other curare-like agents. (See New and Nonofficial Remedies 1955, under the general statement on curare.) Unlike curare and its derivatives, gallamine triethiodide exhibits little action on autonomic ganglions. It is useful with general anesthesia to provide more complete muscular relaxation during surgical, manipulative, endoscopic, and intubation procedures. It is also used to prevent accidents during convulsive shock therapy and to reduce muscle spasm during nonoperative orthopedic procedures. Further observations are needed to confirm its usefulness in obstetrics and for the management of convulsions and chronic spastic states secondary to disease.

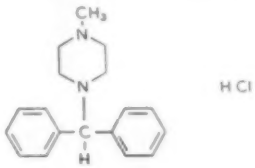
Like all potent curare-like drugs, gallamine triethiodide should be used only by those thoroughly familiar with such agents and only when facilities for intubation, artificial respiration, oxygen therapy, and administration of antidotes are immediately available.

Gallamine triethiodide may produce an allergic reaction in patients sensitive to iodine and care should be taken to avoid severe reactions of this type. The drug is absolutely contraindicated in patients with myasthenia gravis.

Dosage.—Gallamine triethiodide is administered by intravenous injection as an aqueous solution. The dosage should be individualized by careful observation of the patient. The theoretical initial dose is about 1 mg./Kg. of body weight. For prolonged procedures, additional doses of 0.5 to 1 mg./Kg. may be injected at intervals of 40 to 50 minutes. Like curare, its action is cumulative.

The drug is readily miscible with solutions of thiopental sodium. In conjunction with ether inhalation anesthesia, smaller doses are required than for other general anesthetics. The same antagonists that are effective against tubocurarine will interrupt the action of gallamine. Neostigmine methylsulfate, 0.5–1.5 mg., is a useful antidote and atropine sulfate may be administered simultaneously to counteract the postganglionic effect of neostigmine. A larger dose of the antidote is needed when gallamine is used in conjunction with ether because the latter impedes removal of the drug. Antidotes should be used with caution in asthmatic patients sensitive to such drugs. Such adverse antidotal effect is also counteracted with atropine.

CYCLIZINE HYDROCHLORIDE.—Marezine HCl (Burroughs Wellcome).—$C_{18}H_{22}N_2 \cdot HCl$.—M.W. 302.86.—1-diphenylmethyl-4-methylpiperazine hydrochloride.—1-benzhydryl-4-methylpiperazine hydrochloride.—The structural formula of cyclizine hydrochloride may be represented as follows:



Actions and Uses.—Cyclizine hydrochloride is an antihistamine compound that has been found useful in the prevention and relief of motion sickness. Its usefulness as a histamine antagonist in allergic states has not been evaluated. The drug appears to be effective in the control of vertigo, nausea, and vomiting in a high percentage of cases of seasickness and airsickness. Like certain other antihistamines, cyclizine also appears to reduce sensitivity of the labyrinth in the symptomatic relief of vertigo and concomitant symptoms caused by vestibular disorders. The use of the drug for this purpose should not be continued longer than a week without determining the presence or absence of serious vestibular disease or toxic sources of vestibular disturbance for which corrective treatment may be indicated. Its effectiveness for the prevention or treatment of nausea and vomiting from other causes, such as pregnancy and after surgery and anesthesia, has not been established.

In doses ordinarily effective against motion sickness, cyclizine hydrochloride appears to be relatively free of the side-effects usually encountered with antihistamine drugs. High dosage may cause some drowsiness and mouth dryness. The possibility of sedation also should be kept in mind when the drug is used in susceptible persons operating vehicles or machines that require constant alertness.

Dosage.—Cyclizine hydrochloride is administered orally. To prevent and relieve motion sickness, the usual dosage for adults is 50 mg. one-half hour before departure and 50 mg. three times daily before meals. Continued administration of the drug may be unnecessary after the first 2 or 3 days of extended travel, depending on the type of travel, duration of the trip, and susceptibility of a person to motion sickness.

To relieve vertigo and concomitant symptoms of vestibular disorders, the minimum oral dosage for adults is 50 mg. three times daily; twice this amount

(Continued on page VI)

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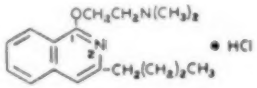
N.N.R. ..... *from page IV*

may be required to completely relieve symptoms.

One-half the adult dosage is suggested for children 6 to 10 years, and one-fourth the adult dosage for those under 6 years of age.

DIMETHISOQUIN HYDROCHLORIDE.—Quotane Hydrochloride (S. K. & F.).—$C_{17}H_{24}N_2O.HCl$.—M.W. 308.86.—3-Butyl-1-(2-dimethylaminoethoxy) isoquinoline hydrochloride.—The structural formula of dimethisoquin hydrochloride may be represented as follows:



Actions and Uses.—Dimethisoquin hydrochloride, an active surface anesthetic, differs chemically from local anesthetics of the benzoate ester type, such as procaine, and is somewhat more active. Its toxicity is less than that of dibucaine but greater than that of cocaine or procaine. Its index of sensitization is considered to be somewhat less than that of procaine derivatives.

Dimethisoquin hydrochloride is useful topically for the relief of itching, irritation, burning, or pain in a wide variety of dermatoses, including nonspecific pruritus and mild sunburn. Evidence indicates that its effectiveness against pruritus is only about 20% greater than that of the vehicle in which it is applied. It also may reduce the pain of sutured surgical wounds. Because of its apparent lack of systemic toxicity and low index of sensitization when applied to the skin, the drug is considered relatively safe for unsupervised use as a topical remedy for symptomatic relief of simple irritations that may accompany undiagnosed minor skin conditions. When these symptoms persist, the underlying cause should be determined by consultation with a physician. Relief of pruritus also may aid in the treatment of the underlying cause with more specific forms of therapy.

Although dimethisoquin hydrochloride has not been associated with systemic toxicity or sensitivity when applied topically to mucous membranes, its use should be restricted to the skin until there has been longer experience regarding its effects on other tissues. For the same reason it should not be applied to extensive areas of the skin. Contact with the eyes should be avoided to prevent stinging.

Dosage.—Dimethisoquin hydrochloride is applied topically to the skin, either as a 0.5% lotion for moist lesions, or as a 0.5% ointment for dry lesions. Either lotion or ointment is applied as a thin film over the affected area. One application of either form usually provides relief for 2 to 4 hours. Application more than four or five times daily seldom is required. Should sensitization appear after repeated applications, further use should be discontinued.

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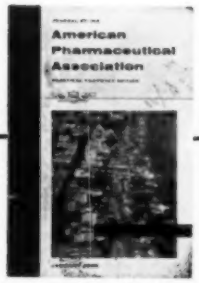

A Preview of Advertising

In the February 1956 issue of the Practical Pharmacy Edition

(All references to actions and uses are taken from the statements of the producers of the products mentioned.)

▶ In its long-familiar, *looked-for* and *looked-at* position on the second cover, **Abbott Laboratories** delineates the "plus" factors of its dietary supplement, **Sur-Bex,** and elsewhere in a two-page spread, describes the efficacy of its antibiotic, **Erythrocin.**

▶ Sage advice is offered by the **American Druggists' Insurance Co.**:—*adjust insurance protection to increasing inventories* to minimize loss when disaster strikes.

▶ **Decholin,** offered by the **Ames Company,** is intended specifically for complaints—common to oldsters—which are related to hepatobiliary and gastro-intestinal functions.

▶ **Span, Tween, Arlacel** and **Sorbo** are some of the adjuncts offered by **Atlas Powder Company** for use in pharmaceutical preparations.

▶ A *new one-inch size* **Vaseline** gauze strip, manufactured by the Professional Products Division of the **Chesebrough Mfg. Co.,** is available through wholesalers or through the Colgate-Palmolive Co.

▶ Indicated for their usefulness in the treatment of diarrhea are **Lactinex** *Granules* and *Tablets,* offered by **Hynson, Westcott & Dunning.**

▶ The **Lederle Laboratories** announcement is given over to "A page of high-profit items worth stocking at all times."

▶ **Lilly** reports that the addition of vitamin B_6 and nicotinamide has increased the *efficacy* of **'Homicebrin'**—but *not* its price.

▶ Offered by **The National Drug Company** are the following "Products of Original Research:" **Parenzyme, Hesper-C, AVC, Protinal, Vi-Protinal, Resion** and **Resion P-M-S.**

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▶ **Abdol,** offered by **Parke, Davis & Company,** supplies 21 vitamins and minerals "to help meet the needs of persons with varied nutritional requirements."

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▶ **Schering's Coricidin,** *a name synonymous with cold control,* "confines the cold without confining the patient."

▶ **Tracinets (Sharp & Dohme)** combine bacitracin, tyrothricin and benzocaine to "help sore throats feel *better, faster.*"

▶ In two insertions, **Smith, Kline & French** (1) announces *a new dosage form* of **Thorazine Suppositories** and (2) adds still another affiant to the many testifying to the sales-effectiveness of its **Benzedrex** inhaler display carton.

▶ The confirmed efficacy of **Cheracol** for coughs is pointed up in **The Upjohn Company's** announcement.

▶ Offered by **Warner-Chilcott** in a new dosage form is **Peritrate** with *Aminophylline*—the efficacy of which is felt "especially in the presence of cardiac and circulatory inadequacy."

▶ In **PNS** (rectal suppositories) and **Milibis** (vaginal suppositories), **Winthrop Laboratories** foresees "More Rx's for You!"

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Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

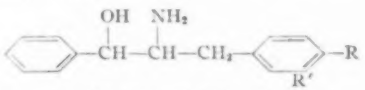
VOLUME XLV — *FEBRUARY 1956* — NUMBER 2 — CONSECUTIVE NO. 4

Alpha,Omega-Diphenyl and *Alpha, Omega*-Cyclohexylphenyl Derivatives of Amino Alkanes and Hydroxy Amino Alkanes*

By WALTER F. CHARNICKI† and JOHN B. DATA

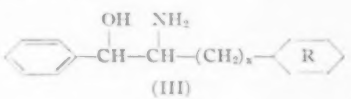
Eight *alpha, omega*-dicarbocyclic derivatives of amino alkanes, four *alpha, omega*-dicarbocyclic derivatives of hydroxy amino alkanes and one *alpha, omega*-dicarbocyclic derivative of amino oxo alkane have been prepared for pharmacological evaluation as depressors.

FROM an investigation of physiologically active amines Algar, Hickey, and Sherry (1) reported that 2-amino-1,3-diphenyl-1-propanol (I) and 2- amino - 3 - (3,4 - methylenedioxyphenyl)-1-phenyl-1-propanol (II)

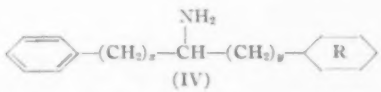


(I), R = H, R′ = H
(II), R,R′ = $-OCH_2O-$

cause a lowering of blood pressure. These investigators state that only when the amino group is adjacent to a hydroxy group on the aliphatic chain is depressor activity observed. The depressor activity reported for I has been confirmed in our laboratories. This observation appeared to warrant additional investigation. Thus, in order to correlate further chemical structure versus physiological activity, 2-amino-1,3-diphenyl-1-propanol (I) has been selected to serve as the prototype for the preparation of a number of related compounds represented by the general formulas III and IV



(III)



(IV)

in which R is phenyl or cyclohexyl and x and y range from 0 to 3, inclusive.

Four compounds of the general formula III were prepared and studied in order to learn in a relative way the depressor effect which results in lengthening the aliphatic chain between the two carbocyclic structures while maintaining the hydroxyl group *alpha* and the amino group *beta* to one of the rings. Eight compounds with the general formula IV have been prepared in order to observe the effect resulting from the lack of a hydroxyl group in compounds of general

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formula III. Incidental to this work a ketone corresponding to a compound of general formula III has been studied.

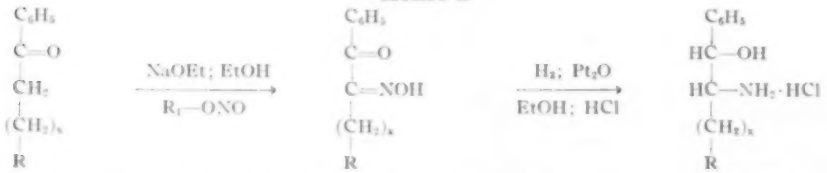
The compounds reported here were prepared according to one of four schemes as illustrated in methods A, B, C, D. In *Method A* a suitable ketone was allowed to react with an alkyl nitrite in the presence of alcoholic sodium ethoxide to yield the nitroso derivative which in turn was reduced catalytically using platinum oxide in an alcoholic solution of hydrochloric acid. In *Method B* a suitable ketone was subjected to the Leuckart synthesis as described by Ingersoll, *et al.* (2), to give the desired amine. In *Method C* an α,α-disubstituted acetic acid was allowed to react with sodium azide and the azide thus obtained was converted to the amine salt by the Curtius reaction. In *Method D* the ketone was brominated and the bromine derivative converted to the amine with liquid ammonia. Methods used to prepare ketones and acids as intermediates are described in *Experimental*.

All compounds prepared were subjected to a blood pressure screening study. All showed depressor activity in the rat. At the dose level used, however, only 2-amino-1,3-diphenyl-1-propanol and 2-amino-1,3-diphenyl-1-propanone hydrochloride showed any prolonged effect, an average of about thirty minutes. All the other compounds prepared produced a very transient fall in blood pressure.

DISCUSSION

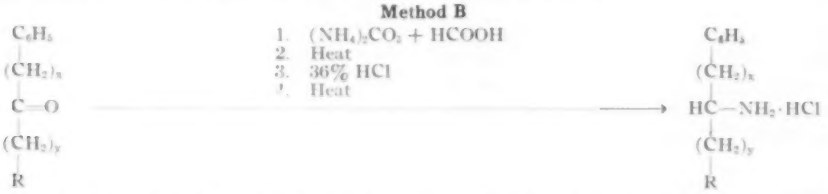
From the blood pressure screening study, it appears that lengthening the aliphatic chain between the two carbocyclic structures while maintaining the hydroxyl group *alpha* and the amino group *beta* to one of the carbocyclic structures tends to decrease the ability of the compounds to lower blood pressure for a prolonged period in the rat. They do, however, cause a transitory fall. It also appears that removal of the hydroxyl group gives compounds which exert only a very transient lowering of blood pressure. In the case of 1-amino-1,3-diphenylpropane, 1-amino-3-cyclohexyl-1-phenylpropane, 1-

Method A



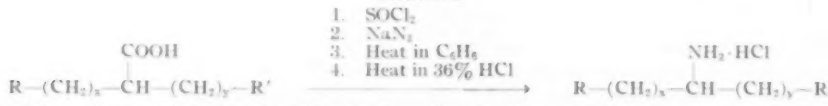
R is phenyl, x is 0 to 2; R is cyclohexyl, x is 1; and R_1 is ethyl or *n*-amyl.[1]

Method B



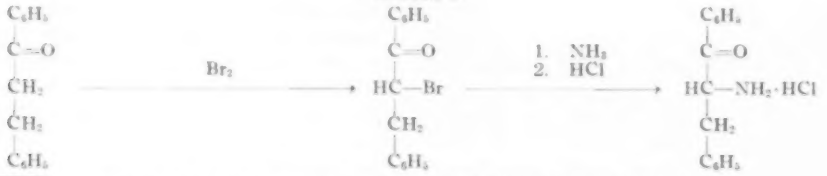
R is phenyl, x is 0 and y is 1,2, or 3; R is phenyl, x and y are 2; R is cyclohexyl, x is 0 and y is 2; and R is cyclohexyl, x is 2 and y is 0.

Method C



R and R' are phenyl, x is 1 and y is 1; R and R' are phenyl, x is 1 and y is 2.

Method D



[1] *n*-Amyl nitrite was used only for the preparation of 2-amino-1,2-diphenyl-1-ethanol hydrochloride.

amino-1,4-diphenylbutane, and 1-amino-1-cyclohexyl-3-phenylpropane hydrochloride it was followed by a pressor effect. Lengthening of the aliphatic chain between the two carbocyclic structures and removal of the hydroxyl group appeared to increase the toxicity of the compounds and decrease the depressor activity. Finally, replacement of a phenyl group by a cyclohexyl appeared to decrease the activity. In the case of 2-amino-1,3-diphenyl-1-propanone hydrochloride, the blood pressure lowering property compares favorably with compound I. It is of interest to note that all of the compounds prepared possess some depressor activity in the test animal.

EXPERIMENTAL

All melting points are uncorrected. Nitrogen was determined by a semimicro Kjeldahl method (3). Unless otherwise stated reduced pressure implies 12 to 16 mm. pressure obtained with a water aspirator. No attempt was made to improve yields obtained from the first trial.

Preparation of 2-Amino-1,2-diphenyl-1-ethanol Hydrochloride.—To a solution of 15.0 Gm. (0.08 mole) of desoxybenzoin [prepared by the Friedel-Crafts synthesis as described by Allen, *et al.* (4)], and 1.8 Gm. (0.08 gram-atom) of sodium dissolved in 60 ml. of absolute alcohol, there was added 9.0 Gm. (0.08 mole) of freshly prepared α-amyl nitrite. The reaction mixture was allowed to stand for two days and then poured into 150 ml. of water. The mixture was extracted with 50 ml. of ether and the ethereal solution was concentrated to an oily residue. The residue was dissolved in a solution of 4 ml. of hydrochloric acid (36%) in 100 ml. of alcohol, 0.2 Gm. of platinum oxide added, and the mixture reduced in a Parr hydrogenator in a hydrogen atmosphere with an initial pressure of 50 p. s. i. After filtering off the catalyst the solvent was removed under reduced pressure and the residue recrystallized several times from isopropyl alcohol to give 3.0 Gm. (17.5%) of product, m. p. 234–235°; reported m. p. 234° (5).

Preparation of 2-Amino-1,3-diphenyl-1-propanone Hydrochloride.—To a solution of 5.3 Gm. (0.025 mole) of benzylacetophenone [prepared according to the procedure described by Adams, Kern, and Shriner (6)], in 50 ml. of anhydrous carbon tetrachloride, there was added dropwise 3.4 Gm. (0.025 mole) of bromine. After the solution had decolorized, the solvent was removed under reduced pressure and the residue dissolved in 50 ml. of anhydrous benzene. Liquid ammonia, 8.5 Gm. (0.5 mole), was added to the benzene solution, the solution placed in a tightly stoppered bottle and the reaction mixture allowed to stand at room temperature for four days. The reaction mixture was exhaustively extracted with 2 *N* hydrochloric acid, and the combined acid extracts were made alkaline with 2 *N* sodium hydroxide. The amine was extracted from the solution with several 50-ml. portions of ether. The combined ethereal solutions were dried over anhydrous sodium sulfate and the hydrochloride prepared by passing dry hydrogen chloride into the filtered ethereal solution. The product was recrystallized from isopropyl alcohol several times to give 0.9 Gm. (13.8%) of product, m. p. 210.5–212° with decomposition.

Anal.—Calcd. for $C_{15}H_{16}ClNO$: N, 5.35. Found: N, 5.01, 5.19.

Preparation of 2-Amino-1,3-diphenyl-1-propanol Hydrochloride.—(*a*) *Isonitrosobenzylacetophenone* was prepared by a modification of the method of Schneidewind (7) from 10.5 Gm. (0.05 mole) of benzylacetophenone, 1.1 Gm. (0.05 gram-atom) of sodium, and 3.8 Gm. (0.05 mole) of ethyl nitrite in 150 ml. of absolute alcohol. The ethyl nitrite was freshly prepared and purified by passing the vapors through a potassium hydroxide tower prior to its use. The reaction mixture was allowed to stand at 0–5° for eighteen hours and then saturated with gaseous carbon dioxide. The solution was filtered. The filtrate was concentrated to about 50 ml. and then poured into 500 ml. of ice-water mixture. The precipitate was filtered off, dried, and then recrystallized from alcohol to give 6.7 Gm. (55.2%) of product, m. p. 124–125°; reported (7), m. p. 125–126°.

(*b*) *2-Amino-1,3-diphenyl-1-propanol hydrochloride* was prepared from isonitrosobenzylacetophenone according to the method described by Algar, Hickey, and Sherry (1). A solution of 3.7 Gm. (0.015 mole) of isonitrosobenzylacetophenone, 100 ml. of alcohol, and 5 ml. of hydrochloric acid (36%) was shaken in the presence of 0.2 Gm. of platinum oxide in a Parr hydrogenator in a hydrogen atmosphere with an initial pressure of 50 p. s. i. When the reduction was completed the catalyst was filtered off and the filtrate concentrated to dryness to give a residue of 2-amino-1,3-diphenyl-1-propanol hydrochloride. The solid was recrystallized from alcohol-ethyl acetate several times to give 2.2 Gm. (52.4%) of pure product, m. p. 247–248° with decomposition; reported m. p. 250° with decomposition (1).

Preparation of 2-Amino-3-cyclohexyl-1-phenyl-1-propanol Hydrochloride.—(*a*) *3-Cyclohexyl-1-phenyl-1-propanone* was prepared from 3-cyclohexanepropionyl chloride following the usual procedure applied in the Friedel-Crafts reaction. The product obtained from 78.1 Gm. (0.5 mole) of 3-cyclohexanepropionic acid and 35.0 Gm. (0.25 mole) of phosphorus trichloride was allowed to react with benzene in the presence of 37.5 Gm. (0.3 mole) of anhydrous aluminum chloride to give the desired product which when recrystallized from methyl alcohol melted at 40–40.5°. The yield was 75.6%.

The semicarbazone was prepared from the ketone and recrystallized several times from ethyl alcohol (95%) to give a product which melted at 163–163.5°.

(*b*) *3-Cyclohexyl-2-isonitroso-1-phenyl-1-propanone* was prepared in a 97.0% yield from 3-cyclohexyl-1-phenyl-1-propanone in a manner similar to that described for the preparation of isonitrosobenzylacetophenone. The impure solid material was recrystallized from alcohol several times to give a product which melted at 148–148.5°.

Anal.—Calcd. for $C_{15}H_{19}NO_2$: N, 5.71. Found: N, 5.59, 5.54.

(*c*) *2-Amino-3-cyclohexyl-1-phenyl-1-propanol hydrochloride* was prepared from 3-cyclohexyl-2-isonitroso-1-phenyl-1-propanone following the procedure described for the preparation of 2-amino-1,3-diphenyl-1-propanol hydrochloride. From 0.05 mole of ketone there was obtained a 37.0% yield of product which melted at 190–191° with decomposition. The compound was recrystallized from isopropyl alcohol to give a pure product which melted at 200–200.5° with decomposition.

Anal.—Calcd. for $C_{18}H_{24}ClNO$: N, 5.19. Found: N, 5.28, 5.13.

Preparation of 2-Amino-1,4-diphenyl-1-butanol Hydrochloride.—(*a*) *4-Phenylbutyryl chloride* was prepared from 104.0 Gm. (0.6 mole) of 4-phenylbutyric acid and 130.3 Gm. (1.18 moles) of thionyl chloride in the usual manner for the preparation of acid chlorides to give 85.0 Gm. (77.5%) of product, b. p. 123–124° at 13 mm. pressure; reported b. p. 119° at 9 mm. pressure (8).

(*b*) *1,4-Diphenyl-1-butanone.*—To 12.2 Gm. (0.5 gram-atom) of dry magnesium turnings in 100 ml. of anhydrous ether, there was added 78.5 Gm. (0.5 mole) of phenyl bromide dissolved in 250 ml. of anhydrous ether in the usual manner for the preparation of a Grignard reagent.

To the cooled Grignard reagent in an atmosphere of nitrogen there was added 47.6 Gm. (0.27 mole) of cadmium chloride with vigorous stirring. The mixture was stirred and refluxed for two and one-half hours at which time a negative Gilman test for a Grignard reagent was obtained (9). After the ether was removed and replaced with 300 ml. of anhydrous benzene, the mixture was refluxed for one and one-half hours. To the cooled solution there was added 77.0 Gm. (0.4 mole) of 4-phenylbutyryl chloride, the mixture refluxed for one hour, and then hydrolyzed with sufficient sulfuric acid (20%) to give two distinct layers. The organic layer and the benzene layer extractions were combined and then washed with 100 ml. of water, 100 ml. of sodium carbonate (5%), 100 ml. of water, and 100 ml. of saturated sodium chloride solution, successively. The solution was dried over anhydrous sodium sulfate, concentrated under reduced pressure and the residue fractionated to give 57.2 Gm. (56.3%) of product, b. p. 143–145° at 0.6 mm. pressure; reported b. p. 199–200° at 18 mm. pressure (10). The compound crystallized upon standing to give a product which melted at 58–59°.

(*c*) *2-Isonitroso-1,4-diphenyl-1-butanone* was prepared from 24.2 Gm. (0.1 mole) of 1,4-diphenyl-1-butanone, 2.5 Gm. (0.1 gram-atom) of sodium and 60.6 Gm. (0.8 mole) of ethyl nitrite following the method described previously for the preparation of isonitrosobenzylacetophenone. After several recrystallizations from anhydrous thiophene-free benzene the pure product melted at 119–120° and weighed 11.0 Gm. (43.5%).

Anal.—Calcd. for $C_{18}H_{18}NO_2$: N, 5.53. Found: N, 5.45, 5.51.

(*d*) *2-Amino-1,4-diphenyl-1-butanol hydrochloride* was prepared in the manner as described above for the preparation of 2-amino-1,3-diphenyl-1-propanol hydrochloride. From 12.0 Gm. (0.04 mole) of 2-isonitroso-1,4-diphenyl-1-butanone there was obtained 3.5 Gm. (30.0%) of product which when recrystallized from isopropyl alcohol melted at 209–209.5° with decomposition.

Anal.—Calcd. for $C_{18}H_{20}ClNO$: N, 5.04. Found: N, 5.18, 5.24.

Preparation of 2-Amino-1,3-diphenylpropane Hydrochloride.—(*a*) *Dibenzylacetic acid* was prepared from diethyl dibenzylmalonate [b. p. 226–227° at 12 mm. pressure; obtained in 50.5% overall yield from diethyl malonate following the procedure of Adams and Kamm (11)]. Saponification with alcoholic potassium hydroxide, then acidification with sulfuric acid followed by decarboxylation of 129.0 Gm. (0.38 mole) of the ester gave 83.2 Gm. (90.1%) of product, m. p. 85–86°; reported m. p. 85–86° (12).

(*b*) *Dibenzylacetyl Chloride* was prepared from 25.0 Gm. (0.1 mole) of dibenzylacetic acid and 23.8 Gm. (0.2 mole) of thionyl chloride following the usual procedure. The excess thionyl chloride was removed under reduced pressure, a number of 50-ml. portions of dry benzene was added and distilled off after each addition until the last trace of thionyl chloride was removed. The residue was dissolved in 75 ml. of cold acetone and then used as such in the next step.

(*c*) *2-Amino-1,3-diphenylpropane hydrochloride* was prepared by reacting the cold acetone solution of dibenzylacetyl chloride with a cold solution of 7.2 Gm. (0.1 mole) of sodium azide in 20 ml. of water. The mixture was kept in an ice bath for fifteen minutes, 100 ml. of cold water added, and the organic residue was extracted with several portions of benzene. The benzene solution was dried by azeotropic distillation under reduced pressure. To the residue there was added 50 ml. of anhydrous benzene and then the solution was refluxed for two hours. There was then added 50 ml. of hydrochloric acid (36%) previously saturated with hydrogen chloride and the solution refluxed for two hours. The solution was concentrated to dryness under reduced pressure. The residue was recrystallized several times from isopropyl alcohol to give 3.8 Gm. (15.4%) of product, m. p. 205–205.5°; reported m. p. 205° (13).

Preparation of 2-Amino-1,4-diphenylbutane Hydrochloride.—(*a*) *2-Benzyl-4-phenylbutyric acid* was prepared from diethyl benzyl-β-phenylethylmalonate [b. p. 193–194° at 1.3 mm. pressure; obtained in 67.7% overall yield from diethyl malonate following the procedure of Adams and Kamm (11)]. Repeating the procedure used for the preparation of dibenzylacetic acid there was obtained 39.2% of product, b. p. 192–193° at 0.7 mm. pressure; reported 230° at 8 mm. pressure (8). The distillate crystallized upon standing to give a product which melted at 55–56°; reported m. p. 59–61° (8).

(*b*) *2-Benzyl-4-phenylbutyryl chloride* was prepared from 25.4 Gm. (0.1 mole) of 2-benzyl-4-phenylbutyric acid and 23.7 Gm. (0.2 mole) of thionyl chloride following the usual procedure. An acetone solution of the crude product for use in the next step was obtained following the directions previously described for dibenzylacetyl chloride.

(*c*) *2-Amino-1,4-diphenylbutane hydrochloride* was prepared in the same manner described for the preparation of 2-amino-1,3-diphenylpropane hydrochloride.

The cold acetone solution of 2-benzyl-4-phenylbutyryl chloride obtained above was allowed to react with 7.2 Gm. (0.11 mole) of sodium azide and the azide in turn was converted to the amine hydrochloride to give 10.6 Gm. (40.6%) of a solid which melted at 180–183°. The crude product was recrystallized from isopropyl alcohol several times to give a product which melted at 184.5–185°, reported m. p. 184–185° (14)[3].

Preparation of 1-Cyclohexyl-3-phenyl-1-propanone.—(*a*) *Cyclohexanecarboxylic Acid* was prepared

[3] The hydrochloride was reported in the literature after completion of this work. The analysis is not reported here in view of this prior publication.

by addition of solid carbon dioxide to the Grignard reagent prepared from 163.0 Gm. (1.0 mole) of cyclohexyl bromide and 24.3 Gm. (1.0 gram-atom) of magnesium turnings. Subsequent hydrolysis gave 63.0 Gm. (49.2%) of product, b. p. 120–121° at 13 mm. of pressure; reported 115–117° at 13 mm. pressure (15).

(*b*) *Cyclohexanecarbonyl chloride* was prepared by reacting 63.0 Gm. (0.5 mole) of cyclohexanecarboxylic acid with 118.9 Gm. (1.0 mole) of thionyl chloride in the usual manner for the preparation of acid chlorides to give 58.0 Gm. (81.0%) of product, b. p. 67–67.5° at 14 mm. pressure; reported b. p. 76° at 17 mm. pressure (15).

(*c*) *1-Cyclohexyl-3-phenyl-1-propanone* was prepared in the manner described above for the preparation of 1,4-diphenyl-1-butanone. From 12.2 Gm. (0.5 gram-atom) of dry magnesium turnings, 92.5 Gm. (0.5 mole) of β-phenylethyl bromide, 46.6 Gm. (0.27 mole) of anhydrous cadmium chloride, 58.0 Gm. (0.4 mole) of cyclohexanecarbonyl chloride, there was obtained 23.6 Gm. (27.3%) of product, b. p. 135–136° at 0.85 mm. pressure.

The oxime prepared from the ketone was recrystallized from ethyl alcohol (50%) several times to give a product which melted at 99–100°.

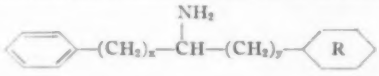
Anal.—Calcd. for $C_{15}H_{21}NO$: N, 6.05. Found: N, 6.09, 6.05.

The semicarbazone prepared from the ketone was recrystallized from ethyl alcohol (95%) several times to give a product which melted at 120–121°.

Preparation α,ω-Diphenyl and α,ω-Cyclohexylphenyl Amino Alkanes.—All compounds listed in Table I were prepared by the Leuckart synthesis according to the procedure reported by Ingersoll, *et al.* (2), as described below except for a few products in which case the molar quantities of the reagents were changed but the molar ratio remained the same.

To 10.7 Gm. of commercial ammonium carbonate there was added cautiously 11.5 Gm. (0.2 mole) of formic acid (85–90%). When the reaction had moderated the temperature was slowly raised to 165°, 0.05 mole of ketone added, and the temperature of the reaction mixture maintained between 165–185° until it became homogeneous (3–8 hrs.). The reaction mixture was cooled, twice its volume of water added, and the oily layer separated off and then refluxed for six hours with 75–100 ml. of hydrochloric acid (36%). The solution was concentrated to dryness under reduced pressure and the residue triturated with several 50-ml. portions of

TABLE I.—α,ω-DIPHENYL and α,ω-CYCLOHEXYLPHENYL DERIVATIVES OF AMINO ALKANES



R	x	y	Yield	Hydrochloride m. p. °C.	Analyses, N, % Formula	Calcd.	Found	
Phenyl	0	2	48.4%	195–196.5[a]	.....	..	..	..
Phenyl	0	1	83.1%	245–246[b]	.....	..	..	..
Phenyl	2	2	40.0%	155–156[c]	$C_{17}H_{22}NCl$	5.07	5.12	5.31
Cyclohexyl	0	2	23.8%	214–215[d]	$C_{15}H_{24}NCl$	5.51	5.63	5.69
Phenyl	0	3	30.5%	177–178[e]	$C_{16}H_{20}NCl$	5.35	5.35	5.57
Cyclohexyl	2	0	24.6%	132–133[f]	$C_{15}H_{24}NCl$	5.51	5.74	5.57

[a] Recrystallized from methyl ethyl ketone; reported (19), m. p. 195°.
[b] Recrystallized from isopropyl alcohol; reported (20), m. p. 251°.
[c] Recrystallized from isopropyl alcohol; reported (14), m. p. 158°.
[d] Recrystallized from ethyl alcohol–ethyl acetate.
[e] Recrystallized from ethyl alcohol–ethyl acetate; reported (14), m. p. 177°.
[f] Recrystallized from isopropyl alcohol.

Preparation of 3-Phenylpropionitrile.—This product was prepared according to the method described by Rupe and Glenz (16) using 90.0 Gm. (0.5 mole) of β-phenylethyl bromide and 90.0 Gm. (1.4 moles) of potassium cyanide to give 51.0 Gm. (77.8%) of product, b. p. 141–142° at 15 mm. pressure; reported b. p. 128–130° at 13 mm. pressure (16).

Preparation of 1,5-Diphenyl-3-pentanone.—This product was prepared according to the general method for the preparation of ketones as described by Hickinbottom (17).

The Grignard reagent was prepared from 74.0 Gm. (0.4 mole) of β-phenylethyl bromide and 9.7 Gm. (0.4 gram-atom) of magnesium in 200 ml. of anhydrous ether. To the Grignard reagent there was added 13.1 Gm. (0.1 mole) of 3-phenylpropionitrile in 150 ml. of anhydrous benzene. After a five-hour reflux period the mixture was hydrolyzed with 82.0 Gm. of hydrochloric acid (36%) and then the anhydrous ether. The solid was recrystallized from an appropriate solvent.

PHARMACOLOGY

To determine whether the compounds prepared possess depressor activity, blood pressure screening studies were made. Healthy albino rats of either sex, Wistar strain, and weighing between 200 and 300 Gm., were anesthetized intraperitoneally with urethane 1.5 Gm./Kg. and prepared for cannulation of the carotid artery. A small bore, 8-mm. mercury manometer was utilized to record the blood pressure changes. Aqueous solutions of the hydrochlorides were prepared and injected as 1% solutions into the common iliac vein at a dose level of 10 mg./Kg. Each compound was tested at this dosage in three individual animals. To prevent cumulative action, one animal was used but for one dose

and then discarded. No attempt was made to determine the degree of depressor activity but this activity was compared to 2-amino-1,3-diphenyl-1-propanol hydrochloride.

All the compounds prepared showed definite depressor activity in the rat. At the dose level used, however, only 2-amino-1,3-diphenyl-1-propanol hydrochloride showed any prolonged effect, an average of about thirty minutes. All the other compounds prepared produced a very transient fall in blood pressure.

SUMMARY

1. Eight α,ω-diphenyl and α,ω-cyclohexylphenyl derivatives of amino alkanes have been prepared.

2. Four α,ω-dicarbocyclic derivatives of hydroxy amino alkanes have been prepared.

3. One α,ω-diphenyl derivative of amino oxo alkane has been prepared.

4. The methods utilized in the preparation of these compounds have been described.

5. Preliminary blood pressure screening indicates that all of the compounds prepared possess some depressor activity when administered to the rat.

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The Tryptophane-Nicotinic Acid Relationships and Alkaloid Biosynthesis in Certain Solanaceae*

By M. C. ANDRIES, J. W. FAIRBAIRN†, and H. W. YOUNGKEN, Jr.

Tryptophane feedings in concentrations of from 0.1 Gm. to 0.6 Gm. per liter were found to stimulate nicotinic acid formation in tomato and *Datura stramonium*. However, no direct effect could be found on Datura alkaloid formation. In the latter case there was evidence that some relationship existed between nicotinic acid levels and those of alkaloid. As nicotinic acid decreased alkaloid increased and vice versa.

IT HAS BEEN PROPOSED by Beadle (1), Nason (2), Gustafson (3), and others (4) that tryptophane can be utilized as a precursor in the biosynthesis of nicotinic acid (niacin) in several organisms including some higher plants. Winterstein and Trier (5) suggested earlier that the pyrrole alkaloid, nicotine, might be formed *in vivo* from proline and nicotinic acid. Dawson (6) has reported larger increases in nicotine with nicotinic acid fed to tobacco plants than with proline feedings.

Other alkaloids of the Solanaceae family such as hyoscyamine and atropine, are condensed pyrrolidine and piperidine derivatives and in this respect possess structural groups which might be formed from precursors common to nicotine (7). However, the relationships between tryptophane and nicotinic acid biosynthesis and the formation of other Solanaceae alkaloids have not been established (8). The objective of this study was to determine by means of feeding experiments with tryptophane what relationship this compound might have on nicotinic acid formation in tomato plants of one variety and on both nicotinic acid and alkaloid in *Datura stramonium*.

EXPERIMENTAL

Healthy *Datura stramonium* and tomato (variety Marglobe) plants were grown from seed in soil. Upon reaching about 12 cm. in height these were carefully transplanted to nutrient culture media in $1^1/_2$-liter culture bottles which were continuously

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aerated from an air line. The nutrient culture liquid consisted of the formula of Hoagland and Arnon (9) adjusted to a *p*H of 5.4–5.5 with potassium in place of the ammonium cation in the phosphate of the nutrient. In some cases nitrate nitrogen was withheld from the nutrient during the tryptophane feedings. Only plants that were employed for excised leaf feedings were grown in soil media. Tryptophane, as *l*-tryptophane, was used in concentrations of 0.1, 0.2, and 0.6 Gm. per liter. This was added to hydroponic cultures when Datura and tomato plants reached from about 50 cm. to 66 cm. in height and were approximately 88 and 97 days old. Leaf material was analyzed for nicotinic acid by the methods of Snell and Wright (10) and "Methods of Vitamin Assay" (11) during which lactic acid production of *Lactobacillus arabinosus* 17-5 was titrated with 0.1 *N* NaOH using bromothymol blue as an internal indicator for a measurement of growth.

Attempts were made to measure tryptophane utilization in terms of its disappearance from culture solutions. The *p*-dimethylaminobenzaldehyde color reaction in the method of Nason (12) was compared electrocolorimetrically with standardized controls. Attempts were also made to measure the tryptophane in plant extractive material according to the same method. It was found, however, that tryptophane deteriorated rather rapidly in a four-day period in the Hoagland nutrient in which no plants were grown (controls.) Losses were about 7% in twenty-four hours, 30% in forty-eight hours, 60% in seventy-two hours, and about 100% at the end of a four-day period. It was therefore concluded that quantitative measurements for tryptophane uptake would be invalid since factors such as oxidation and bacterial contamination in the nutrient media would render results extremely inaccurate. Accordingly it might be assumed that whatever tryptophane was used by plants would need to be taken up within the first two or three days following feedings in order to have any effect *per se* on the metabolic pathway. This, of course, does not rule out the possibilities that tryptophane in fractions of the amounts used was not absorbed as such but was taken up in the form of breakdown products. Further study of this aspect is being pursued.

Total alkaloids in Datura leaves were measured as hyoscyamine according to the method of Witt *et al.* (13) during which one-gram (or less) samples were employed. This method is a modification of the Vitali-Morin procedure.

Tryptophane-Nicotinic Acid Relationships in Tomato Plants.—Fifty-eight young tomato plants were arranged into four groups of about 15 plants per group. Two groups were fed concentrations of tryptophane (0.2 Gm./L. and 0.6 Gm./L.) and nitrate nitrogen was withheld from the culture. One additional group was used as the control for nitrogen deficiency and one control group was fed the full nutrient formula. Plants fed with 0.6 Gm./L. tryptophane showed severe wilt at the end of eight hours and were therefore not continued. Remaining plants showed no toxic effects and were harvested forty-eight hours later. Results of the nicotinic acid (niacin) analyses are shown in Table I.

From these results, comparison of Lot B and Lot D shows that about 19% increase in niacin occurred with tryptophane feedings. Although a similar comparison of control Lot A with control Lot D (no nitrate nitrogen) shows a slight increase in the niacin of the controls with nitrogen, this was not thought to be significant. Lot C is not comparable in that the high tryptophane concentration fed (thrice that of Lot B) was toxic and plants were therefore harvested at an earlier period. However, in the short period of these treatments a niacin increase was indicated. No alkaloid was found in any of the tomato plants.

TABLE I.—THE EFFECT OF TRYPTOPHANE FEEDING AND NIACIN ACCUMULATION IN TOMATO PLANTS AFTER 48 HOURS

Lot	No. of Plants	Nutrient Solution	Dry wt., Gm.	Niacin, μg./Gm.	S. D.
A	13	Control	6.06	51.5	3.4
B	15	0.2 Gm./L. tryptophane, no NO_3	6.54	58.0	3.2
C[a]	15	0.6 Gm./L. tryptophane, no NO_3	5.37	54.0	2.9
D	15	Control, no NO_3	6.11	49.0	3.1

[a] Harvested after 8 hours and analyzed.

It appeared that nitrogen starvation for forty-eight hours caused only slight loss in plant niacin but the tryptophane feedings (Lot B) showed enough increase in niacin over the presence or absence of nitrate nitrogen to be also considered a stimulating factor in niacin accumulation.

Tryptophane-Niacin and Alkaloid Relationships in *Datura stramonium*.—Thirty-one young *Datura stramonium* plants were arranged in five groups and fed concentrations of 0.1 Gm./L. and 0.2 Gm./L. of tryptophane in full nutrient culture for a two-day growth period. One group of seven plants was fed 0.2 Gm./L. tryptophane for a five-day growth period. All plants including nontreated controls were then measured for niacin and total alkaloid formation. The results are shown in Table II and Fig. 1.

From these results it appeared that there was a significant difference between niacin and alkaloid formation in the untreated two- and five-day controls. Niacin decreased and alkaloid increased. In the five-day growth period alkaloid increased by

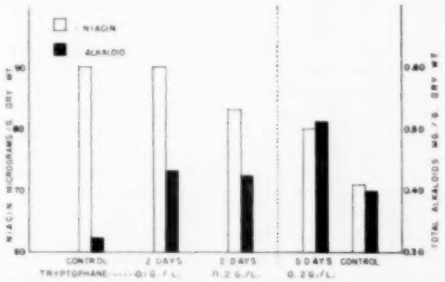

Fig. 1.—Tryptophane-niacin and alkaloid formation in *Datura stramonium* leaves.

TABLE II.—TRYPTOPHANE-NIACIN FEEDINGS AND ALKALOID FORMATION IN DATURA STRAMONIUM LEAVES

No. plants	Nutrient Solution	Dry wt., Gm.	Niacin µg./Gm.	Niacin S.D.	Total Alkaloids mg./Gm.	Total Alkaloids S.D.
4	Control, 2-day	10.12	90	2.52	0.32	0.038
5	Control, 5-day	15.51	71	2.41	0.40	0.029
9	0.1 Gm./L. tryptophane, 2 days	23.00	90	2.00	0.43	0.015
6	0.2 Gm./L. tryptophane, 2 days	15.33	83	2.35	0.42	0.016
7	0.2 Gm./L. tryptophane, 5 days	21.71	80	2.72	0.51	0.021

TABLE III.—TRYPTOPHANE-NIACIN AND ALKALOID FORMATION IN SOIL-GROWN EXCISED TOMATO AND STRAMONIUM LEAVES[a]

Leaves[b]	Tomato Dry wt., Gm.	Tomato Niacin µg./Gm.	Tomato Niacin S.D.	Stramonium Dry wt., Gm.	Stramonium Niacin µg./Gm.	Stramonium Niacin S.D.	Stramonium Total Alkaloids mg./Gm.	Stramonium Total Alkaloids S.D.
U. Control	7.41	56	3.2	7.13	49	3.6	2.58	0.38
L. Control	3.90	44	2.8	5.98	62	3.4	2.03	0.32
U 0.25 Gm./L. tryptophane (4 days)	4.59	63	4.1	6.04	52	2.9	2.49	0.37
L. 0.25 Gm./L. tryptophane (4 days)	8.23	49	3.4	6.47	63	3.1	2.00	0.35

[a] Leaves selected by random sampling of 22 tomato and 15 stramonium plants, 14 weeks old.
[b] U—upper leaves. L—lower leaves.

TABLE IV.—TRYPTOPHANE-NIACIN AND ALKALOID FORMATION IN DATURA UNDER AERATION AND NON-AERATION CONDITIONS FOR FIVE DAYS

Lot	No. Plants	Aerated Solution	Dry wt., Gm.	Niacin µg./Gm.	Niacin S.D.	Alkaloids mg./Gm.	Alkaloids S.D.
A	6	Control in dist. H_2O	39.8	80	3.6	1.10	0.025
B	7	0.2 Gm./L. tryp., dist. H_2O	42.3	60	3.1	0.72	0.038
C	7	0.6 Gm./L. tryp., dist. H_2O	45.57	55	2.3	0.93	0.023
		Nonaerated Solution					
D	6	Control in dist. H_2O	37.6	63	2.7	0.77	0.034
E	7	0.2 Gm./L. tryp., dist. H_2O	37.5	67	3.8	0.54	0.011
F	7	0.6 Gm./L. tryp., dist. H_2O	34.0	57	1.9	0.86	0.027

about 25% whereas niacin decreased by about 21%. In the two-day growth period of the tryptophane-treated plants a somewhat similar result was noted in alkaloid accumulation when compared with non-treated plants. In this case an alkaloid increase of about 33% occurred but in the case of those plants to which 0.2 Gm./L. of tryptophane was fed, a decrease of only about 8% in niacin was evident. Perhaps the most significant observation is that in a five-day growth period with tryptophane there was about 27% increase in alkaloid, while instead of a decrease in niacin there was approximately a 12% increase. From the latter observation it might be reasoned that some competition existed between alkaloid and niacin for the utilization of tryptophane but that the total gain was in favor of alkaloid. On the other hand this could also be interpreted as a significant gain in niacin from the tryptophane and that alkaloid was related inversely to nicotinic acid levels. The next two experiments were devised to test both hypotheses.

Excised Leaf Experiments.—In order to assess the effects of tryptophane on niacin and alkaloid it appeared that more rapid absorption would take place in excised tomato and Datura leaves. The leaves of fourteen week-old plants were therefore excised and immersed in a water solution of 0.25 Gm./L. of tryptophane for four days. The petioles of upper (U) younger leaves and lower (L) older leaves were employed. Aeration was withheld from these solutions and leaves were then collected and analyzed. Results are shown in Table III and Fig. 2.

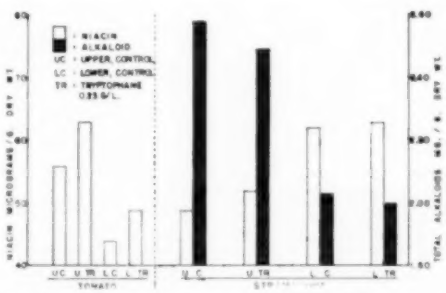

Fig. 2.—Tryptophane-niacin and alkaloid formation in soil-grown excised tomato and stramonium leaves.

From these results there appeared to be no significant increase in niacin formation in either kind of leaf material. The total alkaloid of Datura leaves in nontreated and treated conditions was significantly higher (more than four times) than previously noted during hydroponic culture feedings. However, this was attributed to the soil conditions in which plants had been grown prior to excision and not to tryptophane feedings since untreated plants showed approximately the same high alkaloid formation. It would seem that since tryptophane did not produce the same effects on niacin and alkaloid in excised leaves as was noted for intact plants, other factors present in root tissues were necessary for its optimum utilization. For example,

the conversion of this compound to kynurenin, hydroxyanthranilic acid, proline, and similar intermediates of nicotinic acid as proposed by Rosen (14) and Beadle (15) obviously require complete enzyme systems of root and leaf. It is possible that this complete system was missing or significantly inhibited under conditions of root and leaf separation.

In order to determine what effects tryptophane might have on niacin and alkaloid in the leaves of intact Datura plants when grown in the absence of other components of the culture solution, an experiment was arranged employing straight water culture to which tryptophane was added. Forty plants were grown in distilled water, six and seven plants per group, and fed 0.2 Gm. and 0.6 Gm./L. with and without aeration for a period of five days. Results of this experiment are shown in Table IV.

In this case the control plants which received aeration produced the most niacin and alkaloid. In the two instances of aeration during which tryptophane was added in two different concentrations (0.2 and 0.6 Gm./L.) with the higher tryptophane concentration there was concomitantly a significant increase over the lower concentration in alkaloid but this did not reach the level of the aerated controls. This was similarly observed in Lot E of the nonaerated plants. To some extent aeration favored alkaloid formation in all plants even though it had previously been shown that tryptophane should not be expected to remain stable under conditions of aeration.

Conversely under conditions of nonaeration in which tryptophane should not deteriorate as rapidly, there should be more stimulation of alkaloid formation if such a relationship existed. However, the results in Table IV generally do not indicate this. As niacin decreased, alkaloids increased and in one case (Lot E) as the niacin increased the alkaloid decreased. It would seem, therefore, that alkaloid accumulation was more directly related to niacin decrease or increase than to any direct stimulation by tryptophane.

CONCLUSION

It can be concluded from this study that there is evidence for the stimulation of niacin production in tomato plants from tryptophane feedings and this also occurred, but less conspicuously, in *Datura stramonium*. On the other hand, in general a Datura alkaloid increase also resulted, but since in these instances this was more directly related to a niacin decrease it would appear that the effects of tryptophane on alkaloid were indirectly through its influence on niacin.

SUMMARY

1. By direct feeding tryptophane was found to increase the total niacin formation in Marglobe tomato plants.

2. In Datura a decrease in niacin occurred during the growth period of untreated (control) plants but this decrease was significantly less in two conditions when plants were under the influence of tryptophane. Generally, the alkaloid accumulations in Datura appeared to be more directly related to niacin levels than to direct tryptophane stimulation. As niacin decreased alkaloid increased and vice versa.

3. Excised leaf material from soil-grown plants were similarly compared for the effects of tryptophane. No significant niacin or alkaloid increases were observed. However, approximately a fourfold increase in total alkaloid production was found to occur in soil-grown Datura plants when compared with plants grown in the nutrient culture.

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A New Approach to the Assay of Alkaloidal Crude Drugs I.*

Cinchona and Nux Vomica

By EINAR BROCHMANN-HANSSEN

A new approach to the assay of alkaloidal crude drugs is described. The method, which involves a simple ion exchange technique, gives results which are 10–15 per cent higher than those obtained by the conventional methods. It is, furthermore, rapid, has a high degree of precision, and requires little skill on the part of the operator.

THERE HAS BEEN little progress in the field of alkaloidal assays of crude drugs since Keller (1) in 1894 developed the general scheme which is still used in the majority of the official methods. Most of the work that has been done since that time, has been directed toward developing better methods for the quantitative estimation of the pure alkaloids. Relatively little attention has been paid to the extraction and purification steps of the assays. It seems rather unlikely that significant improvements can result from mere modifications of the older types of methods presently official in the various pharmacopeias. A radically new approach to the problem is necessary, making use of recent developments in separation and purification techniques, as well as modern methods for the quantitative determination of the isolated alkaloids.

In a previous publication, the author has reported that inorganic salts which are practically insoluble in water, rapidly undergo ion exchange when shaken with an aqueous suspension of a strongly acidic cationic exchange resin (2). This observation has led to the idea that such a resin may be used in the same way to extract alkaloids from crude drugs. After the alkaloids have been transferred from the crude drug to the ion exchange resin, it would seem to be a relatively simple matter to recover the alkaloids from the resin by elution with alcoholic ammonia.

Cinchona and nux vomica are two crude drugs which always have presented great problems, both with regard to the extraction as well as the purification and the determination of the extracted alkaloids. Experiments with these drugs have revealed that the simple ion exchange technique indicated above actually gives a more complete extraction in a shorter time than the conventional maceration methods.

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Presented to the Scientific Section, A. PH. A., Miami Beach meeting, May 1955
After the experimental work reported in this paper was nearly completed, J. Büchi and F. Furrer in *Arzneimittel-Forsch.*, **3**, 1(1953); **4**, 307(1954), described a similar ion exchange technique for extraction of cinchona.

EXPERIMENTAL

Reagents and Equipment.—Dowex® 50–X_2, 50–100 mesh, Dowex® 1–X_1, 50–100 mesh, 2.5 *N* HCOOH, 95% C_2H_5OH, 4 *N* NH_3 in 70% v/v methanol, reference standards for the alkaloids, extraction tubes, ion exchange columns, mechanical shaker, electrical heating tape, and spectrophotometer for ultraviolet spectrophotometry.

The alkaloidal salts used as reference standards were crystallized several times from alcohol, and the content of alkaloidal base and of water was determined. The solutions prepared from these salts were calculated in terms of the concentration of anhydrous alkaloid base.

Ion Exchange Extraction.—In order to achieve a rapid extraction of the crude drug and a quantitative recovery of the adsorbed alkaloid with a minimum of eluant, it was necessary to use a cationic exchange resin of high porosity. Dowex® 50–X_1 and Dowex® 50–X_2 were found to be the most satisfactory in this respect. Dowex® 50–X_1 is the more porous of the two and actually gave a somewhat more rapid extraction at room temperature. Nevertheless, we preferred to use Dowex® 50–X_2 because its greater density facilitated the subsequent removal of the crude drug residue. The ion exchange resin was activated with 5 *N* hydrochloric acid in a chromatographic tube and then washed with water until the filtrate was neutral to methyl red. The excess of water was removed by suction, but the resin was not allowed to dry out. It was stored in a closed container.

When 25 ml. of an aqueous solution of an alkaloidal salt, corresponding to 5–10 mg. of the base, was shaken for ten minutes with 1 Gm. of the activated ion exchange resin, the alkaloid was quantitatively adsorbed by the resin. It could be recovered by elution with 50–100 ml. of 4 *N* NH_3 in 70% v/v methanol. In applying this method to cinchona, it was observed that the major portion of the alkaloids (about 80%) was extracted in ten to fifteen minutes. It required several hours, however, before the yield became constant. The rate of extraction could be increased by raising the temperature and by adding small amounts of formic acid. The optimum acid concentration appeared to be in the neighborhood of 0.1 *N*. The temperature used was 80–90°. Under these conditions 0.1 Gm. of finely powdered cinchona

was completely extracted in thirty minutes, while the same amount of nux vomica required only fifteen minutes for complete extraction. The extraction tube used in these experiments is pictured in Fig. 1. Its total capacity was about 50 ml. Heat was applied by means of an electrical heating tape wrapped around the tube. The voltage necessary to give the desired temperature was found in a blank experiment and maintained by means of an autotransformer. The tube was clamped horizontally in a mechanical shaker (Eberbach) in such a way that the glass stopper was held firmly in place.

Purification.—After the extraction was complete, the mixture was transferred to an ion exchange column, where the crude drug residue was removed by a backwash technique, leaving the heavier ion exchange resin in the column. The ion exchange column used for this purpose is illustrated in Fig. 2. The bent tube from the 3-way stopcock was connected to a distilled water outlet for the purpose of backwashing. The bulb in the middle of the column facilitated the separation of the crude drug residue from the resin.

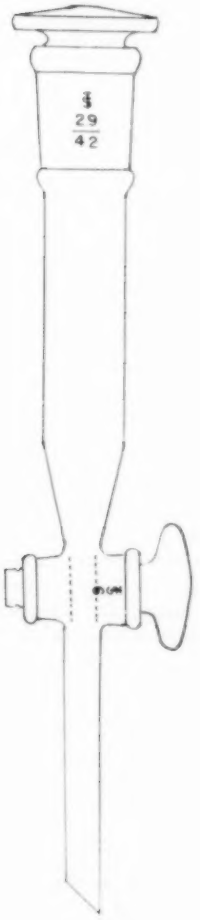

Figure 1.

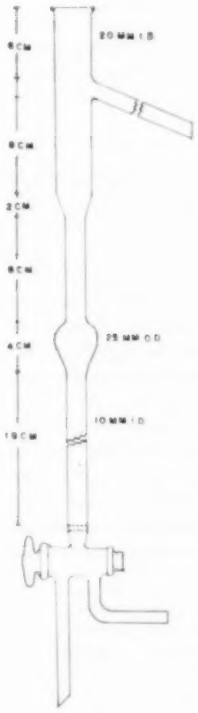

Figure 2.

Considerable amounts of colored, nonalkaloidal impurities were adsorbed on the cationic exchange resin during the extraction period. Some of these impurities could be removed by washing with alcohol, but the major portion was eluted together with the alkaloids with alcoholic ammonia. It was observed that a very efficient purification could be accomplished simply by passing the eluate from the cationic resin directly through a highly porous anionic resin of the quaternary ammonium type. The non alkaloidal impurities were adsorbed on the anionic resin, whereas the alkaloids passed through unhindered. Dowex® 1-X_1 (Cl^-) was used for this purification. It was interesting to note that those impurities which could be removed from the cationic resin by washing with alcohol, were not as readily adsorbed by the anionic resin as were the others.

Quantitative Estimation.—The alkaloids could be determined directly in the eluate by ultraviolet spectrophotometry. The ultraviolet absorption spectra for the cinchona alkaloids have been studied by a number of workers at different *p*H values (3–6). Although the optical isomers have the same spectra, there are sufficient differences in the absorption spectra of the two main structural types of alkaloids to permit a separate determination of the quinine-quinidine fraction and the cinchonine-cinchonidine fraction. This has considerable advantage over a determination of total alkaloids, because there often is a great variation in the ratio of these two fractions depending on variety, location, etc. The amount of alkaloids in the sample was calculated from the following simultaneous equations: $D_1 = C_q E_{q1} + C_c E_{c1}$; $D_2 = C_q E_{q2} + C_c E_{c2}$ where D_1 and D_2 are the absorbances at 332 mμ and 280 mμ, respectively; E is E (1%/1 cm.); and C_q and C_c are the concentrations of the quinine-quinidine fraction and the cinchonine-cinchonidine fraction in the sample solution.

Strychnine and brucine can also be determined separately in a mixture by ultraviolet spectrophotometry (7, 8), thus eliminating the necessity for oxidizing brucine. The absorbance of the ammoniacal eluate was determined at 304 and 260 mμ. The amount of the alkaloids was calculated in the same way as shown above for cinchona, substituting in the equations the appropriate values for the absorbances of strychnine and brucine.

Analytical Procedure.—Place 0.1 Gm. of finely powdered cinchona or nux vomica, accurately weighed, in an extraction tube, add 1 ml. of 2.5 *N* formic acid, 1 Gm. of activated Dowex® 50-X_2, and 25 ml. of boiling distilled water. Shake the mixture in a mechanical shaker for thirty minutes for cinchona, or fifteen minutes for nux vomica, while maintaining a temperature of 80–90°. Transfer the mixture quantitatively to a suitable ion exchange tube and remove the crude drug residue by means of backwashing. Allow the water to drain off down to the surface of the resin and wash the resin with 25 ml. of 95% ethanol. Remove the alcohol and air bubbles, which have formed in the resin layer, by a brief wash and backwash combination, and elute the alkaloids from the resin with 4 *N* NH_4 in 70% v/v methanol. Let the eluate flow directly through a column of Dowex® 1-X_1 (Cl^-) (50–100 mesh), which previously has been washed with

about 50 ml. of alcoholic ammonia. A column of Dowex® 1-X_1 which is 1 cm. in diameter and 15 cm. in height, can be used for two samples of cinchona or for numerous samples of nux vomica. Collect 100 ml. of eluate in a volumetric flask, and determine the alkaloids by ultraviolet spectrophotometry, using blanks which have been obtained by passing methanolic ammonia through the same column of Dowex® 1-X_1.

It is well known that the official gravimetric assay of cinchona does not lead to a pure alkaloid residue. The weight of this residue, therefore, does not give a true picture of its alkaloidal content. This was shown experimentally in the following way: The alkaloidal residue obtained according to the N. F. procedure was dissolved in a little alcohol. One-tenth normal hydrochloric acid was added in an amount calculated to convert the alkaloids to the monohydrochlorides, and the solution was diluted to 1000 ml. with water. Twenty-five milliliters of this solution was shaken for fifteen minutes with 1 Gm. of Dowex® 50-X_4 (H^+), and the alkaloids were eluted with methanolic ammonia as described above. The results of this experiment, as well as the results of the official assay and of the new ion exchange method, are shown in Table I.

TABLE I.—ASSAY OF CINCHONA[a]

	Quinine + Quinidine, %	Cinchonine + Cinchonidine, %	Total Alkaloid, %
N. F. method	....	....	6.45 ± 0.03
N. F. extraction with ion exchange purification	2.48 ± 0.02	3.87 ± 0.01	6.35 ± 0.03
Ion exchange extraction and purification	2.87 ± 0.02	4.17 ± 0.02	7.04 ± 0.03

[a] The results are average values of at least three determinations.

The official assay of nux vomica is based on a determination of strychnine after brucine has been removed by oxidation with nitric acid and sodium nitrite. It would be of interest to know just how complete the removal of brucine is, and to what extent strychnine is affected by this treatment. With this in mind, samples of the crude drug were extracted according to the official procedure, and the alkaloids purified as described in the N. F., with and without oxidation of brucine. The alkaloidal residues were neutralized with a calculated amount of 0.1 *N* hydrochloric acid and diluted to a suitable volume with water. Twenty-five milliliters of this solution was extracted with the cationic exchange resin as described above for cinchona. The results are given in Table II.

CONCLUSIONS

From the experiments described it may be concluded that it is possible to obtain what appears to be a complete extraction of cinchona and nux vomica, as well as a satisfactory purification of the alkaloids by means of a simple ion exchange technique. The results obtained by this method are about 10–15% higher than those obtained by the official procedures. By removing the impurities from the alkaloidal residues obtained in the official cinchona assay, a better basis for comparison between the relative efficiencies of the two extraction procedures is achieved.

Nux vomica is somewhat easier to extract than cinchona, and the extraction of nux vomica by the official procedure is, therefore, not quite as incomplete as in the case of cinchona. It has been shown, however, that a considerable amount of strychnine is lost during the oxidation of brucine. In the proposed method, this oxidation is eliminated, and the two alkaloids are determined by ultraviolet spectrophotometry.

TABLE II.—ASSAY OF NUX VOMICA[a]

	Strychnine, %	Brucine, %	Total Alkaloid, %
N. F. method	1.21 ± 0.02	....	....
N. F. extraction, oxidation of brucine, ion exchange purification	1.18 ± 0.02	0.04 ± 0.01	....
N. F. extraction, ion exchange purification	1.30 ± 0.01	1.58 ± 0.02	2.88 ± 0.02
Ion exchange extraction and purification	1.38 ± 0.01	1.72 ± 0.02	3.10 ± 0.02

[a] The results are average values of at least three determinations.

SUMMARY

A new approach to the assay of alkaloidal crude drugs has been described. The alkaloids are extracted by shaking the powdered crude drug with an aqueous suspension of a strongly-acidic, cationic exchange resin. The crude drug residue is removed by a backwash technique, and the alkaloids are eluted with methanolic ammonia. The eluate is purified by passing through a highly porous, strongly basic anionic exchange resin, and the alkaloids are determined by ultraviolet spectrophotometry.

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Composition of Gum Turpentines of Pines XXV.*

A Report on two White Pines: *Pinus koraiensis* from Korea and *P. peuce* from Macedonia

By P. M. ILOFF, Jr. and N. T. MIROV

As a whole, turpentine of *P. koraiensis* had the following composition: *dl, l*-α-pinene, 29 per cent; *l*-β-pinene, 15 per cent; Δ³-carene, 7 per cent; *dl, l*-limonene, 6 per cent; *n*-undecane, trace; *dl, l*-bornyl acetate, 2 per cent; sesquiterpenes, mostly longifolene, 9 per cent; diterpenes, 20.5 per cent; oxygenated compounds, 1 per cent; residue and losses, 10.5 per cent. As a whole, turpentine of *P. peuce* had the following composition: *dl, l*-α-pinene, 50 per cent; *l*-β-pinene, 6 per cent; β-myrcene, 2 per cent; terpinolene, 1 per cent; *dl, l*-bornyl acetate, 1 per cent; two sesquiterpenes, one of which is of a cadalene type, 9 per cent; diterpenes, mainly cembrene, 17 per cent; oxygenated compounds, probably diterpene alcohols, 2 per cent; residue and loss, 12 per cent.

PINUS KORAIENSIS

Pinus koraiensis Sieb. et Zucc. is a white pine growing in the mountains of Manchuria, Korea, and the central part of the Japanese Island of Honshu. Two samples of oleoresin of the pine were obtained from Seoul, Korea; one from Dr. S. K. Hyun of the Department of Forestry, Seoul National University, and another from Dr. Min Sim Hyun, Director of the National Forest Experiment Station, Seoul, Korea. Their assistance in obtaining the samples is highly appreciated.

EXPERIMENTAL

The oleoresin, amounting to 2,800 Gm., was heated under reduced pressure and the turpentine recovered. Turpentine distilled below 190° at 0.1 mm. of mercury, and designated as light oil, amounted to 18% of the weight of the oleoresin. At this stage, the pot residue was still soft and tacky. When the temperature was increased to 200° and pressure decreased to 0.05 mm. of mercury, a heavy oil started to distill. Some of it solidified in the condenser. This heavy oil amounted to 9% of the weight of the oleoresin. Physical properties of both light and heavy oil are given in Table I.

TABLE I.—PHYSICAL PROPERTIES OF TURPENTINE FROM OLEORESIN OF *PINUS KORAIENSIS*

Fractions	Per cent of the Weight of Oleoresin	Density d_4	Index of refraction n_D	Specific Rotation $[\alpha]_D$
Light oil	18	0.8634^{25}	1.4730^{25}	−11.6
Heavy oil	9	0.9300^{23}	1.5213^{20}	+119.0

A 433-Gm. sample of the light oil was fractionated with a 90-cm., 25-mm. inside diameter Todd column packed with 3/32-inch single-turn glass helices. A reflux ratio of 10:1 was maintained. The results of the fractionation are shown in Table III.

The physical properties of the first three fractions were close to those of α-pinene. A 7-ml. portion of fraction 2 was used to prepare *dl*-α-pinene nitrosochloride which, after two recrystallizations, amounted to 1.2 Gm. It melted at 103.5–104.5° and showed no depression in melting point when mixed with an authentic sample of *dl*-α-pinene nitrosochloride. The *dl*-α-pinene nitrosochloride was converted to *dl*-α-pinene-nitrolpiperidide which, after purification, melted at 120–121° and showed no melting point depression when mixed with authentic *dl*-α-pinene nitrolpiperidide.

Judging from the physical properties, fraction 4 was a transition fraction containing both α- and β-pinenes.

Fractions 5, 6, and 7 were mainly *l*-β-pinene. An alkaline permanganate oxidation of 20 Gm. of fraction 6 yielded 1.0 Gm. of crude nopinic acid which, after two recrystallizations from benzene, melted at 125–126° and showed no melting point depression on admixture with authentic nopinic acid.

* Received May 10, 1955, from the Institute of Forest Genetics, California Forest and Range Experiment Station, maintained by the Forest Service, U. S. Department of Agriculture, in cooperation with the University of California, Berkeley.

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Fraction 8 was a transition fraction containing both β-pinene and Δ^3-carene.

Fractions 9, 10, and 11 contained mainly *d*-Δ^3-carene. From 5 ml. of fraction 10, 0.15 Gm. of Δ^3-carene nitrosate, melting at 145°, was prepared. From fraction 11, Δ^3-carene nitrosate melting at 143–144° was prepared. The melting point was not depressed by admixture of authentic Δ^3-carene nitrosate.

Fractions 12 and 13 were transition fractions containing both *l*- and *dl*-limonene and *d*-Δ^3-carene. Fractions 14 and 15 contained mainly *l*- and *dl*-limonene.

From 2 ml. of fraction 15, 0.4 Gm. of solid tetrabromide was prepared. After several recrystallizations from ethyl acetate at 0° the derivative melted at 124–125° and was not depressed in melting point by admixture of authentic dipentene tetrabromide.

Fractions 16 and 17 were transition fractions containing both limonene and *n*-undecane. The physical constants of fractions 18 and 19 indicated the possible presence of *n*-undecane.

From 6.38 Gm. of fractions 18, 19, and 20, 2.0 Gm. of oil was obtained which was insoluble in 18% fuming sulfuric acid. The physical constants of the oil recovered after repeated shaking with fuming sulfuric acid, compared with those for *n*-undecane, were:

Constant	*n*-Undecane from *P. koraiensis*	*n*-Undecane[1]
n_D	$1.4167^{22.3}$	1.41902^{20}
d_4	0.7364^{23}	0.7404^{20}
b.p.	196–197[760]	195.8[760]

[1] Egloff, G., "Physical Constants of Hydrocarbons," Vol. I, Am. Chem. Soc. Monograph No. 78, Reinhold Publishing Corp., N. Y., 1939, p. 76.

Analysis of undecane from *P. koraiensis:*

Anal.—Calcd. for $C_{11}H_{24}$: C, 84.52; H, 15.48. Found: C, 84.98; H, 15.15.

Fraction 21 had a high density, and the odor suggested the presence of bornyl acetate. A 2.9-Gm. portion of fraction 21 was saponified with aqueous sodium hydroxide. From the alcohol portion, 0.74 Gm. of crude *p*-nitrobenzoate was prepared. After recrystallization from alcohol its melting point was 153.5–154°. The *dl*-bornyl *p*-nitrobenzoate, prepared by Shaw (4), melted at 152–154°. A *dl*-bornyl *p*-nitrobenzoate, prepared by mixing equal weights of the *p*-nitrobenzoate of *d*- and of *l*-borneol, melted at 153–154° and did not depress the melting point of the above sample of *dl*-bornyl *p*-nitrobenzoate derived from fraction 21. A *p*-bromophenacyl ester melting at 82–83° was prepared from the acid portion of the ester. A *p*-bromophenacyl ester of acetic acid did not depress the melting point of the above derivative.

Fractions 22, 23, and 24 were mixtures of *l*- and *dl*-bornyl acetate and sesquiterpenes. Fractions 25 to 29 contained sesquiterpenes, the major component being *d*-longifolene.

From fraction 26, 0.5 Gm. of solid sesquiterpene hydrochloride was obtained. After two recrystallizations from glacial acetic acid, the hydrochloride melted at 58.5–59°, and its melting point was not depressed on admixture of authentic longifolene hydrochloride from *d*-longifolene.

To sum up, the light oil of *P. koraiensis* contains: *l*- and *dl*-α-pinene, 44%; *l*-β-pinene, 22%; *d*-Δ^3-carene, 11%; *l*- and *dl*-limonene, 8.5%; *n*-undecane, 0.5%; *l*- and *dl*-bornyl acetate, 3%; sesquiterpenes (mainly *d*-longifolene), 7.4%, and pot residue and loss, 3.6%.

A 156-Gm. sample of the heavy oil was distilled through a 3-inch Vigreux column at reduced pressure. The results of the distillation are shown in Table III.

Fractions A to D were mostly sesquiterpenes containing some diterpene. Fractions E to M all contained diterpene hydrocarbons, and each one crystallized on standing in the refrigerator when seeded. Fractions G to L were filtered, yielding 31.9 Gm. of crude diterpene. A second crop of 3.8 Gm. was obtained. The 31.9-Gm. portion recrystallized from ethanol, yielded 18.8 Gm. of crystals which melted at 57–59°. After a second recrystallization from ethanol the crystals melted at 58–59°, had an optical rotation $[\alpha]_D^{23}$, +236°, c = 2.40 in chloroform. On admixture with cembrene from *P. peuce*, the melting point was not depressed.

Three grams of the combined filtrate of fractions G to L was treated with one Gm. of maleic anhydride and shaken vigorously at 100° until well mixed, then heated at 100° for thirty minutes. After cooling, the mixture crystallized and was washed on the filter with hexane. The remaining crystals were recrystallized, and 1.7 Gm. was received. After another recrystallization from glacial acetic acid, the melting point was 148–149° and was not depressed by admixture with the similar adduct from fraction H of the heavy oil of *P. peuce* which, as mentioned later in this paper, was found to be the same as a diterpene maleic anhydride adduct derived from *P. armandi* (3). The fractions M and N are mostly diterpene with some oxygenated compounds. Fraction O probably contains considerable amounts of diterpene alcohol.

Analysis of fraction O: Found: C, 84.34; H, 11.51.

To sum up, the heavy oil of *P. koraiensis* contained: sesquiterpenes, 12%; diterpenes, 60–61% (mainly cembrene and including a diterpene having a maleic anhydride adduct m. p. 148–149°); oxygenated compounds, 3%; pot residue and loss 24–25%.

As a whole, turpentine of *P. koraiensis* (i. e., both light and heavy oils combined) had the following composition: *dl*, *l*-α-pinene, 29%; *l*-β-pinene, 15%; Δ^3-carene, 7%; *dl*, *l*-limonene, 6%; *n*-undecane, trace; *dl*, *l*-bornyl acetate, 2%; sesquiterpenes, mostly longifolene, 9%; diterpenes (mostly cembrene and including a diterpene having a maleic anhydride adduct, m. p. 148–149°), 20.5%; oxygenated compounds, 1%; residue and losses, 10.5%.

PINUS PEUCE

Pinus peuce Grisebach is a rare white pine of southeastern Europe, morphologically similar to *Pinus griffithii* (*P. excelsa*) of India (5). It grows at elevations from 2,500 to 6,000 feet in the mountains of Macedonia and in adjacent parts of Albania, Bulgaria, and Greece. A sample of *Pinus peuce* oleoresin was obtained through the courtesy of Prof. Dr. Branislav Pejoski of the University of Macedonia, Skopje, Macedonia, Yugoslavia. Pejoski himself had investigated oleoresin of this pine, and steam-distilled turpentine had the following characteristics: Yield, 19 to 22%; density, d^{20},

0.861 to 0.866; index of refraction, n_D^{20}, 1.462 to 1.463; specific rotation $[\alpha]_D$, −13.33 to −29.36°.

EXPERIMENTAL

The oleoresin used in the present investigation was heated under reduced pressure. At a maximum temperature of 180° and pressure 0.5 mm. of mercury, 23.3% of turpentine distilled over. This is designated in Table II as fraction 1. After this operation, the pot residue (rosin) was still very soft on cooling. When temperature was increased to 200–205° and pressure reduced to 0.05 mm., about 9.5% more distillate was obtained. This portion of the oil was designated as fraction 2. It had the consistency of castor oil and was of yellowish color and faint fragrance. Similar behavior in distillation was previously experienced with oleoresins of *Pinus albicaulis* (2), *P. parviflora* (1), *P. koraiensis* (reported earlier in this article), and *P. armandi* (3).

Physical characteristics of *P. peuce* turpentine (if one could call the second portion of the distillate by this name) are given separately in Table II for each portion of distillate. It should be noted that when Pejoski used steam distillation for obtaining turpentine of *P. peuce*, only the first portion was distilled over; the second one remained in the still. Under these conditions, rosin is generally soft and tacky.

A batch of 655 Gm. of the turpentine was fractionated with a 90-cm. long, 25-mm. inside diameter Todd column packed with 3/32-inch single-turn glass helices. A reflux ratio of 10:1 was maintained. The results of the distillation are shown in Table III.

The properties of fraction 1 are close to those of α-pinene. From 7 ml. of fraction 1, 1.8 Gm. of *dl*-α-pinene nitrosochloride was prepared. After recrystallization, it melted at 104–105° and showed no depression of its melting point on admixture with authentic *dl*-α-pinene nitrosochloride. The nitrolpiperidide, m. p. 120–121°, was also prepared from the nitrosochloride of fraction 1. The melting point of the nitrolpiperidide was not depressed by admixture with an authentic sample of *dl*-α-pinene nitrolpiperidide.

Fraction 2 was mainly *l*- and *dl*-α-pinene containing some β-pinene. Fractions 3 and 4 were transition fractions containing both α-pinene and β-pinene.

Fraction 5 was mostly *l*-β-pinene. The density is low due to the presence of some β-myrcene. From alkaline permanganate oxidation of 20 Gm. of fraction 5, 2.0 Gm. of sodium nopinate was received. The sodium nopinate was converted to nopinic acid which, after recrystallization from benzene, melted at 125–125.5° and did not depress the melting point of authentic nopinic acid.

Fraction 6 was a transition fraction containing both *l*-β-pinene and β-myrcene.

Fractions 7 and 8 had unusually low densities, indicating the possible presence of β-myrcene. One and two-tenths grams of fraction 7 was added to 0.48 Gm. maleic anhydride and warmed on a steam bath. A violent reaction took place. The reaction mixture was taken up in 40 ml. hexane and allowed to stand several days at 0°; 0.4 Gm. of crystals, m. p. 30°, was then filtered off. After recrystallization, the adduct melted at 32–33° and was not depressed in melting point by addition of authentic β-myrcene maleic anhydride adduct.

Fractions 9 and 10 were transition fractions containing both β-myrcene and terpinolene. Fraction 11 had properties close to the properties of terpinolene. Fraction 11 redistilled over sodium, yielded a heart cut with the properties: d_4^{24}, 0.8496; n_D^{24}, 1.4813. From 2 ml. of the heart cut, 1 Gm. of tetrabromide was prepared, which, after several recrystallizations from chloroform by addition of cold methanol, melted at 116–117°, and its melting point was not depressed by admixture of authentic terpinolene tetrabromide.

Fraction 12 was a transition fraction containing both terpenes and bornyl acetate. Fractions 13 and 14 had properties, including odor, which indicated the presence of *l*- and *dl*-bornyl acetate. A 2.8 Gm.-portion of fraction 14 was saponified. From the alcohol portion, a *p*-nitrobenzoate was prepared which, after two recrystallizations from alcohol, weighed 190 mg. and melted at 151–153°. When mixed with the authentic *p*-nitrobenzoate of *dl*-borneol (prepared by mixing the *p*-nitrobenzoate of *d*-borneol with that of *l*-borneol as described above for *P. koraiensis*)[2] the melting point of the *p*-nitrobenzoate was 153–154°. The acid portion of the ester was isolated by acidification followed by steam distillation. The steam distillate was neutralized and reduced in volume to 5 ml. to which 150 mg. of *p*-nitrobenzyl bromide, dissolved in alcohol, was added. After refluxing for one hour, 86 mg. of a derivative melting at 74–77° was isolated; after recrystallization from hexane, the melting point was 77–78° and was not depressed by admixture of authentic *p*-nitrobenzyl ester of acetic acid.

Fraction 26 was a transition fraction containing both bornyl acetate and sesquiterpenes. Fractions 27, 28, and 29 contained mainly a dextrorota-

TABLE II.—PHYSICAL CHARACTERISTICS OF THE LOW-BOILING AND HIGH-BOILING FRACTIONS OF *P. peuce* TURPENTINE

Fractions of distillate	Per cent of the Weight of Oleoresin	d_4	n_D	$[\alpha]_D^{23}$
Fraction 1, 160–180° at 0.5 mm.	23.1%	0.8628^{24}	1.4717^{23}	−16.2
Fraction 2, 200–205° at 0.5 mm.	9.4%	$0.9280^{22.5}$	1.5200^{23}	+113.0
Pajoski's data for steam-distilled *P. peuce* turpentine	19–22%	0.861 to 0.866	1.462 to 1.463	−13.33 to −29.36

[2] Thanks are due to Mr. Gene Kritchevsky of the Forestry Department, University of California for a pure sample of the *p*-nitrobenzoate of *d*-borneol.

TABLE III.—FRACTIONAL DISTILLATION OF TURPENTINES OF *Pinus koraiensis* AND P. *peuce*

Fractions	Pressure, mm.	Boiling Range, °C.	Distillate, %	Density d_4^{24}	Index of Refraction n_D^{25}	Specific Rotation $[\alpha]_D^{25}$
P. koraiensis, light oil (433 Gm. used)						
1	13	43–44	3.7	0.854	1.4631	−19.6
2	13	44–45	30.5	0.852	1.4642	−20.6
3	13	45–47	7.8	0.855	1.4669	−21.8
4	13	47–48	1.9	0.857	1.4704	−22.5
5	13	48–50	2.0	0.860	1.4730	−22.8
6	13	50–50	10.2	0.862	1.4756	−22.5
7	13	50–52	5.8	0.861	1.4760	−21.0
8	13	52–55	4.0	0.856	1.4744	−8.2
9	13	55–56	1.1	0.850	1.4728	0.0
10	13	56–57	1.5	0.847	1.4720	+2.6
11	13	57–58	4.3	0.847	1.4719	−0.7
12	13	58–59	2.5	0.843	1.4723	−19.0
13	13	59–60	1.8	0.841	1.4729	−40.4
14	13	60–64	2.9	0.839	1.4729	−62.3
15	13	64–66	1.0	0.839	1.4736	−63.2
16	13	66–69	1.0	0.838	1.4732	−51.8
17	13	69–72	0.6	0.836	1.4710	−32.3
18	13	72–76	0.7	0.824	1.4635	−11.2
19	13	76–91	0.6	0.811	1.4461	−4.9
20	13	91–99	0.4	0.869	1.4580	−12.0
21	13	99–104.5	0.8	0.954	1.4719	−16.1
22	13	104.5–110	0.9	0.934	1.4779	−10.3
23	13	110–114	0.7	0.929	1.4817	−1.9
24	13	114–118	1.1	0.919	1.4890	+13.3
25	13	118–120	1.9	0.916	1.4957	+23.6
26	13	120–121	1.9	0.916	1.4983	+26.6
27	9	115–116	1.5	0.913	1.4998	+23.7
28	9	116–118	0.5	0.910	1.5001	+20.9
29	9	Above 118	1.8	0.909	1.4005	+12.1
Pot residue and loss	..	.....	4.6			
P. koraiensis, heavy oil (156 Gm. used)				d_4^{23}	n_D^{23}	$[\alpha]_D^{25}$
A	6	99–110	1.7	0.924	1.4933	+14.1
B	6	110–125	7.1	0.917	1.5022	+49.1
C	6	125–130	2.9	0.915	1.5065	+72.2
D	6	130–145	0.6	0.916	1.5052	...
E	6	145–155	1.8	0.913	1.5104	+105.0
F	6	155–165	2.0	0.911	1.5149	+126
G	6	165–170	1.6	0.909	1.5149	+142
H	6	170–175	9.2	0.905	1.5180	+170
I	6	175–177	15.7	..	1.5193	...
J	6	177–178	11.8	..	1.5203	...
K	6	178–179	6.4	0.905	1.5209	+151
L	6	179–180	5.3	0.908	1.5218	...
M	3	177–179	4.7	..	1.5228	...
N	1	155–170	2.4	..	1.5238	...
O	1	Above 170	2.4	..	1.5289	...
Pot residue and loss	..	.....	23.0 1.4			
P. peuce, light oil (655 Gm. used)				d_4^{24}	n_D^{23}	$[\alpha]_D^{25}$
1	12	40.5–41	59.7	0.853	1.4649	−23.4
2	12	41–44	10.1	0.853	1.4666	−26.5
3	12	44–46	1.2	0.856	1.4708	−29.9
4	12	46–48	1.5	0.859	1.4736	−34.2
5	12	48–50	7.3	0.858	1.4750	−33.9
6	12	50–53	0.4	0.835	1.4733	−23.5
7	12	53–55	0.6	0.813	1.4720	−9.4
8	12	55–59	0.4	0.815	1.4737	+3.2
9	12	59–61	0.4	0.829	1.4761	+11.6
10	12	61–66	0.3	0.840	1.4788	+10.5
11	12	66–79	1.0	0.855	1.4820	+4.0
12	12	79–89	0.3	0.886	1.4813	−5.9
13	12	89–95	0.3	0.937	1.4792	−12.8
14	12	95–107	1.1	0.937	1.4783	−13.5
15	12	107–113	0.8	0.935	1.4831	0.0
16	12	113–116	1.0	0.925	1.4912	+10.5
17	12	116–117	1.1	0.918	1.4960	+14.2
18	12	117–119	1.2	0.913	1.4990	+13.0
19	12	119–123	1.4	0.907	1.5000	+4.3
20	12	123–125	1.3	0.903	1.5007	−11.3
21	3	78–80	0.4	0.902	1.5032	−22.0
22	3	80–83	1.0	0.907	1.5042	−0.3

Pot residue	..	.....	3.4			
Loss	..	.....	3.8			
	P. peuce, heavy oil (306 Gm. used)			d_4^{24}	$n_D^{23.4}$	$[\alpha]_D^{24}$
A	10	70–134	1.1	..	1.4962	+35.0
B	6	110–132	2.8	0.9163	1.5002	+37.1
C	6	132–135	2.8	0.9138	1.5023	+41.8
D	6	135–145	2.3	0.9126	1.5047	+54.8
E	6	145–155	2.1	0.9129	1.5080	+76.7
F	6	155–165	4.0	0.9110	1.5123	+112
G	6	165–172	8.0	0.9056	1.5121	+151
H	6	172–174	8.6	0.9044	1.5187	+160
I	6	174–176	11.1	0.9029	1.5198	+160
J	6	176–179	10.9	0.9059	1.5207	+152
K	6	179–182	8.7	0.9081	1.5214	+137
L	6	182–183	4.6	0.9179	1.5227	+102
M	6	183–188	2.3	0.9396	1.5258	+48.8
N	3	180–190	2.7	0.9484	1.5283	+6.3
O	1.5	170–175	3.6	0.9755	1.5325	−10.5
Pot residue	..	.....	22.8			
Loss	..	.....	1.6			

tory sesquiterpene which gave no hydrochloride when treated with dry HCl gas in dry ether. The fractions also gave no aromadendrene color test.

Fraction 32 gave 0.1 Gm. of hydrochloride, m. p. 116.5–117°, from 2.2 ml. of oil. The melting point of the derivative was not depressed by admixture of cadinene hydrochloride, of rotation −40°, in chloroform.

Fraction 33 was redistilled, yielding a heart cut with the following properties: b_4, 118–121°; d_4^{24}, 0.9073; n_D^{24}, 1.5040; $[\alpha]_D^{24}$, +18.1°. From 3 ml. of this oil, 0.3 Gm. of hydrochloride was prepared. The hydrochloride, after two recrystallizations from glacial acetic acid, melted at 116.5–117° and its melting point was not depressed by admixture with cadinene hydrochloride, of rotation −40°, in chloroform. The derivative had the rotation $[\alpha]_D^{23}$, −41.1°; c = 0.9 in $CHCl_3$.

To sum up, the light oil of *P. peuce* oleoresin contained: *dl*,*l*-α-pinene, 71%; *l*-β-pinene, 9%; β-myrcene, 2%; terpinolene, 1.5%; *dl*, *l*-bornyl acetate, 2%; sesquiterpenes 7.4%, mainly consisting of two sesquiterpenes, one of which is a cadalene-type sesquiterpene; and 7.1% pot residue and loss.

A 306-Gm. portion of the heavy oil was distilled at reduced pressure through a 3-inch Vigreux column. The results of the distillation are shown in Table III. Fractions A to D consisted mainly of sesquiterpenes. Fraction C was redistilled over sodium with an 8-inch Vigreux column. A heart cut had the following properties: b_{10}, 127–130°; n_D^{23}, 1.5002; d_4^{24}, 0.912; $[\alpha]_D^{24}$, +19.3°. No crystalline hydrochloride was obtained by the usual treatment with dry HCl gas. Fraction E was a transition fraction, and fractions F to L were predominantly diterpene hydrocarbons. Fraction I:

Anal.—Calcd. for $(C_5H_8)n$: C, 88.17; H, 11.83. Found: C, 88.12; H, 12.00.

Fractions E to L had formed crystals on scratching or seeding while standing at 0°. Twenty milligrams of crystals was filtered from fraction E. After recrystallization from ethanol the crystals melted at 58–59°, and the melting point was not depressed when cembrene from fraction H was added. Six grams of the crystalline diterpene was removed from fraction H by filtration. The diterpene was recrystallized from ethanol, m. p. 58–59°; rotation $[\alpha]_D$, +227°, c = 5 in chloroform. The melting point was not depressed by admixture of cembrene from *P. albicaulis* or *P. armandi* (2, 3).

The properties of the mother liquor of fraction H were: d_4^{23}, 0.910; n_D^{23}, 1.5187; $[\alpha]_D^{23}$, +108°. The mother liquor probably contained considerable amounts of cembrene since it continued to crystallize slowly when standing at 0°.

Three grams of the filtrate from fraction H was treated with 1 Gm. of maleic anhydride, heated to 100°, and shaken vigorously, and then kept at 100° for thirty minutes; after cooling, 3 ml. of hexane was added and 0.76 Gm. of adduct was filtered off. The white crystals were twice recrystallized from glacial acetic acid and finally melted at 148–149°. The melting point was not depressed on admixture with a similar adduct from fraction H of *P. armandi* (3).

Fractions M and N contained diterpenes and probably diterpene alcohols. Fraction O was very viscous; judging from the analysis and the fact that it did not react with ketone reagents, it consisted of one or more diterpene alcohols. Fraction O:

Anal.—Calcd. for $C_{20}H_{34}O$: C, 82.69; H, 11.80. Found: C, 82.56; H, 11.51.

To sum up, the heavy oil of *P. peuce* was found to contain: sesquiterpenes, 10%; diterpenes, mainly cembrene (with a small amount of diterpene with maleic anhydride adduct melting at 148–149°) 59–60%; oxygenated compounds, probably diterpene alcohols, 6%; residue and loss 24%.

As a whole, turpentine of *Pinus peuce* (i. e., both light and heavy oil combined) had the following composition: *dl*, *l*-α-pinene, 50%; *l*-β-pinene, 6%; β-myrcene, 2%; terpinolene, 1%; *dl*, *l*-bornyl acetate, 1%; two sesquiterpenes, one of which is of a cadalene type, 9%; diterpenes, mainly cembrene, 17%; oxygenated compounds, probably diterpene alcohols, 2%; residue and loss, 12%.

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Bacterial Resistance Studies Involving Several Drugs Useful in the Treatment of Urinary Tract Disease*

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Sulfacetamide, methenamine, and mandelic acid were studied in an effort to determine which drug or combination of drugs is the most useful in the treatment of urinary tract disease. Utilizing a described method of analysis, results are reported of attempts made to produce resistant strains of organisms to the three drugs and four combinations of them. Finding the combination of all three drugs to be most useful, an excretion study was made using human subjects.

DUE TO THE development of bacterial fastness to many of the newer antibacterial drugs, methenamine and mandelic acid have taken a more prominent position in modern concepts of therapy in urinary tract disease. Duca and Scudi (1) and Scudi and Duca (2) have reported that methenamine mandelate possesses antibacterial activity comparable to sulfonamides in urinary tract disease, but with the added advantage that the causative organisms have difficulty in becoming fast to these drugs. Duca and Scudi have demonstrated that organisms made resistant to sulfathiazole remained susceptible to methenamine mandelate.

Sulfacetamide has been reported to be among the least toxic but one of the most effective of the sulfonamides against urinary tract pathogens (3–7). Several investigators have reported a simultaneous increase of resistance by the organisms to the sulfonamides after exposure to only one of these drugs (3, 8, 9). Similar reports have been made regarding the antibiotics by Fusillo and Romansky (10) and Herrell, *et al.* (11). However in 1947, Welebir and Barnes (6) reported that certain sulfanilamide-resistant, mandelate-resistant, and sulfathiazole-resistant organisms remained susceptible to sulfacetamide.

This paper reports results of attempts made to produce strains of organisms resistant to sulfacetamide, methenamine, mandelic acid, and various combinations of these drugs, in an effort to determine which of the drugs or combinations possess the widest antibacterial spectrum and the lowest resistance liability.

EXPERIMENTAL

Bacterial Cultures.—The original cultures were recently isolated[1] from the urine of patients afflicted with urinary tract disease. Neither antibiotics nor sulfonamides had been administered for a period of several months prior to the isolation of the organisms. The six organisms selected for study included *Escherichia coli*, *Aerobacter aerogenes*, *Micrococcus pyogenes* var. *aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *beta* hemolytic *Streptococcus pyogenes*.

The culture medium used was modified S-R medium experimental.[2] This semisynthetic liquid medium contains no sulfonamide inhibitors and adequately supports the growth of all organisms used in this study. The medium was modified slightly from the composition as reported by Wilson (12) by the removal of the agar by filtration at room temperature. The medium was adjusted to a *p*H of 5.5 utilizing a Beckman glass electrode *p*H meter. This adjustment was made in order to provide an acid medium which is necessary for the maximum antibacterial activity of methenamine and mandelic acid. Sulfacetamide has been shown to be equally active in both acid and alkaline media. This medium was tubed in 4.5-ml. and 5.0-ml. quantities in 18 x 150-mm. Pyrex test tubes by utilization of an automatic pipet. The medium was freshly prepared and sterilized by autoclaving at 15 pounds pressure for fifteen minutes.

Drugs and Drug Combinations.—Sulfacetamide, methenamine, mandelic acid, and four combinations of these three drugs in the proportions shown in Table I were employed for this study. Stock solutions were maintained with the exception of those containing methenamine, which were freshly prepared just prior to use.

Experiment I.—Sensitivity Tests.—Preliminary tests were made to determine the highest concentrations of each drug and drug combination in which each of the organisms could grow. In performing this test the drug solutions were diluted in such a manner that the exact quantity of drug required to produce the desired concentration was contained in a volume of 0.5 ml. The final volume of all tubes before inoculation was 5.00 ml. Sulfacetamide

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[1] The original cultures were obtained from the bacteriology department of the University Hospital in Oklahoma City, Okla.

[2] This medium was generously and kindly supplied by Difco Laboratories, Detroit, Mich.

TABLE I.—DRUG COMBINATIONS

Combination Number	Drugs	Amounts, Gm.
I	Sulfacetamide	0.25
	Methenamine	0.125
II	Sulfacetamide	0.25
	Mandelic acid	0.136
III	Methenamine	0.125
	Mandelic acid	0.136
IV	Sulfacetamide	0.25
	Methenamine	0.125
	Mandelic acid	0.136

sensitivity tests were made in modified S-R medium both at *p*H 7.4 and *p*H 5.5 without noticeable change of activity.

Each tube was inoculated with one 3-mm. loopful of a twenty-four hour brain-heart infusion (Difco) culture. At the end of a twenty-four-hour incubation period at 37°, the tubes were observed for visible growth to determine the maximum concentration of each drug and drug combination in which growth could occur. The tubes were allowed to remain in the incubator for as long as ninety-six hours without a change of results. The presence of visible growth provided a clearly defined end point

TABLE II.—RESULTS OF ATTEMPTS TO PRODUCE RESISTANCE USING SULFACETAMIDE, METHENAMINE, AND MANDELIC ACID

Tests	*E. coli*	*Aerobacter aerogenes*	*M. pyogenes* var. *aureus*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Streptococcus pyogenes*
Original sensitivity of organisms in mg. % to:						
Sulfacetamide	20.00	18.75	10.00	40.00	10.00	1.25
Methenamine	20.00	5.00	1.25	2.50	0.625	1.25
Mandelic acid	20.0	20.00	30.00	10.00	25.00	1.56
Final sensitivity of organisms in mg. % to:						
Sulfacetamide	70.00	500.00	40.00	175.00	550.00	30.00
Methenamine	30.00	10.00	5.00	10.00	1.25	1.25
Mandelic acid	40.00	50.00	60.00	20.00	40.00	10.00
Number of transfers						
Sulfacetamide	21	21	18	18	12	19
Methenamine	14	8	8	19	10	9
Mandelic acid	12	17	18	14	16	10
Increase of resistance						
Sulfacetamide	3.5×	26.6×	4.0×	55.0×	4.37×	24.0×
Methenamine	1.5×	2.0×	4.0×	4.0×	2.0×	1.0×
Mandelic acid	2.0×	2.5×	2.0×	2.0×	1.6×	6.4×

TABLE III.—RESULTS OF ATTEMPTS TO PRODUCE RESISTANCE TO FOUR COMBINATIONS OF SULFACETAMIDE, METHENAMINE, AND MANDELIC ACID

Tests	*E. coli*	*Aerobacter aerogenes*	*M. pyogenes* var. *aureus*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Streptococcus pyogenes*
Original sensitivity of organisms in mg. %* to:						
Combination No. I	1.25	1.87	2.50	0.625	1.25	0.156
Combination No. II	1.25	10.00	10.00	5.00	1.25	0.625
Combination No. III	5.00	1.56	2.50	0.321	5.00	0.78
Combination No. IV	1.25	4.00	2.50	1.25	2.50	0.625
Final sensitivity of organisms in mg. %* to:						
Combination No. I	5.00	20.00	5.00	10.00	20.00	0.625
Combination No. II	10.00	40.00	20.00	30.00	30.00	5.00
Combination No. III	20.00	10.00	5.00	5.00	20.00	5.00
Combination No. IV	5.00	10.00	5.00	10.00	20.00	1.25
Numbers of transfers						
Combination No. I	19	17	14	18	9	14
Combination No. II	17	15	17	17	16	14
Combination No. III	15	16	10	19	17	14
Combination No. IV	18	16	14	17	15	16
Increase of resistance						
Combination No. I	4×	10.6×	2×	16×	16×	4×
Combination No. II	8×	4×	2×	6×	24×	8×
Combination No. III	4×	6.4×	2×	16×	4×	6.4×
Combination No. IV	4×	2.5×	2×	8×	8×	2×

*Values shown are those of sulfacetamide with other drugs in proportion as shown on Table I.

for the interpretation of these tests. Duplicate tubes were employed as a check in every instance. This procedure was carried out for each organism studied with all three drugs and the four drug combinations. The activity determined by this method was termed the original sensitivity and the results appear in Tables II and III.

Experiment II.—The Production of Drug Resistance.—The production of drug resistance was effected by repeatedly transferring the organisms through increasing concentrations of the drugs in modified S-R medium. The tubes were inoculated with an 8-mm. loopful of a twenty-four-hour brain-heart infusion culture and incubated for twenty-four to seventy-two hours. Periodic subcultures were made in order to rule out the possibility of contamination.

The evaluation of this experiment was made from data found in Tables IV and V. The resistance index shown in these tables may be defined as the ratio of the number of transfers to the increase of resistance brought about by the transfers. The resistance index of a drug is therefore inversely proportional to the ease of the production of resistance. By this definition, a high index appearing in the table indicates that the organism had greater difficulty in developing a resistance than those possessing lower resistance indexes.

The evaluation of the merit of the drugs and the combinations was further aided by the calculation of an efficiency index which included an additional consideration of the final sensitivity, as well as the factors of the resistance index.

$$\text{Efficiency index} = \frac{1}{\text{final sensitivity}} \times \frac{\text{No. of transfers}}{\text{increase of resistance}}$$

Efficiency indexes have been calculated in such a manner that the higher figures represent the greater usefulness against the organisms.

Experiment III.—The Rate of Excretion of the Combination of All Three Drugs.—This experiment was performed in order to obtain an indication of the time required after oral administration to produce antibacterial concentrations of the drugs in the urine and the duration of their activity. Two tablets, each containing sulfacetamide, 0.25 Gm., methenamine, 0.125 Gm., and mandelic acid, 0.136 Gm., were administered orally to five normal adult human subjects. The relative amount of the drugs excreted in the urine was determined by the antibacterial activity of the urine produced by the drugs and was thus assayed microbiologically. A normal urine specimen, taken just prior to the administration of the drug combination, served as a control. Periodic urine specimens were taken throughout a twenty-four-hour period following the administration of the drug. Each urine sample was subjected to measurements of volume and *p*H. Measurements of volume were made for purposes of calculation of the concentrations of the drugs present. The urine output was not found to be significantly altered from normal. Three hours after the administration of the drug combination, the urine was found to be more acidic. After seven hours, the mean *p*H value of the urine samples was found to be 5.39. All samples became sufficiently acid in reaction, due to the presence of excreted mandelic acid, to provide an effective acid medium which is necessary for the therapeutic action of methenamine. In order to provide uniform conditions necessary for microbiological assay, all urine samples were adjusted to *p*H 5.5 with a diluted solution of either hydrochloric acid or sodium hydroxide. The samples were then sterilized by filtration through ultrafine fritted glass bacterial filters. Following the procedure of Experiment I, the tubes were inoculated with one 3-mm. loopful of a twenty-four-hour brain-heart infusion culture of *E. coli* from the original strain. The results thus obtained were

TABLE IV.—RESISTANCE INDEXES OF ALL DRUGS FOR ALL ORGANISMS

Drug or Combination	Organisms						Mean R. I.[a]
	E. coli	*A. aerog.*	*M. pyog.* var. *aur.*	*P. vulg.*	*Ps. aerug.*	*Strep. pyog.*	
I	4.75	1.60	7.00	1.12	0.56	3.50	3.09
II	2.12	3.75	8.50	2.83	0.66	1.75	3.27
III	3.75	2.50	5.00	1.18	4.25	2.18	3.14
IV	4.50	6.40	7.00	2.12	1.87	8.00	4.98
Sulfacetamide	6.00	0.79	4.50	4.11	0.22	0.79	1.73
Methenamine	1.33	4.00	2.00	4.75	5.00	9.00	4.34
Mandelic acid	6.00	6.80	9.00	7.00	10.00	1.56	6.72

[a] Resistance index.

TABLE V.—EFFICIENCY INDEXES OF ALL DRUGS FOR ALL ORGANISMS

Drug or Combination	Organisms						Mean R. I.[a]
	E. coli	*A. aerog.*	*M. pyog.* var. *aur.*	*P. vulg.*	*Ps. aerug.*	*Strep. pyog.*	
I	0.95	0.080	1.40	0.112	0.028	5.60	1.36
II	0.212	0.094	0.43	0.094	0.022	0.35	0.20
III	0.187	0.25	1.00	0.24	0.21	0.44	0.39
IV	0.90	0.64	1.40	0.212	0.093	6.40	1.61
Sulfacetamide	0.086	0.0016	0.11	0.023	0.0004	0.026	0.41
Methenamine	0.31	0.40	0.40	0.48	4.00	7.20	2.13
Mandelic acid	0.15	0.14	0.15	0.35	0.25	0.16	0.20

[a] Efficiency index.

TABLE VI.—THE RATE OF EXCRETION OF SULFACETAMIDE, METHENAMINE, AND MANDELIC ACID IN COMBINATION

Hours After Single Oral Dose	Relative Concentration of the Drugs in the Urine[a]											
	1			3			7			11		
Subject	mg.	mg.	%	mg.	mg.	%	mg.	mg.	%	mg.	mg.	%
Ar	1.10	1.70	2.67	2.50	10.83	6.25	10.00	6.25	3.42	1.78	4.95	1.25
Ed	0.70	1.56	23.07	41.20	8.39	6.25	1.96	2.08	0.94	1.38	4.01	3.75
Al	4.03	11.25	107.66	91.24	10.13	6.25	2.83	1.38	2.73	1.25	4.00	1.25
Am	4.60	1.25	204.35	41.20	37.34	6.25	6.08	3.75	1.62	1.56	1.92	1.39
Ob	1.45	1.25	11.25	11.25	2.43	1.39	3.36	1.39	5.32	1.39	6.00	1.25

Hours After Single Oral Dose	Relative Concentration of the Drugs in the Urine[a]					
	15			24		
Subject	mg.	mg.	%	mg.	mg.	%
Ar	6.25	3.42	1.78	4.95	1.25	
Ed	2.08	0.94	1.38	4.01	3.75	
Al	1.38	2.73	1.25	4.00	1.25	
Am	3.75	1.62	1.56	1.92	1.39	
Ob	1.39	5.32	1.39	6.00	1.25	

[a] Concentrations are expressed in terms of sulfacetamide. Mandelic acid and methenamine are present in concentrations proportional to their rate of excretion in the urine.

compared to the *E. coli* sensitivity tests of Experiment I and a relative activity was determined.

Inasmuch as the drugs are not excreted at exactly the same rate, this procedure does not actually measure the concentration of the individual drugs in the urine. However, a bactericidal activity *versus* time curve can be drawn utilizing data obtained from this experiment. The results illustrate the bactericidal activity of the urine after a single dose of the three drugs in combination and are shown graphically in Table VI, and Fig. 1 and 2.

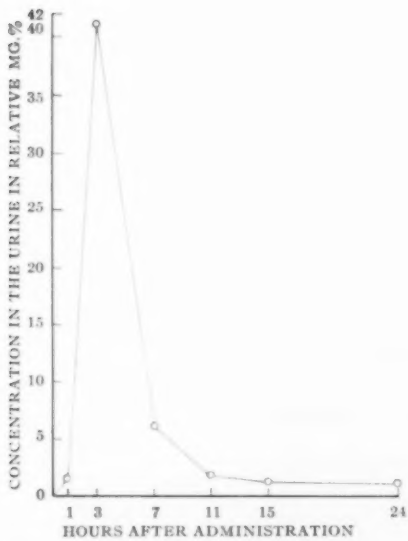

Fig. 1.—Excretion rate of the combination of sulfacetamide, methenamine, and mandelic acid in relative mg.%. This curve represents the antibacterial activity of the drugs excreted in the urine after the single administration of the combination, as determined by culturing *E. coli* in dilutions of the urine containing the excreted drugs. For purposes of calculation, it was assumed that the urine contained the same proportional quantities of each drug as it did when administered. Consequently, only the values of sulfacetamide were plotted and concentrations of methenamine and mandelic acid may be assumed to be present in the proportions indicated on Table 1.

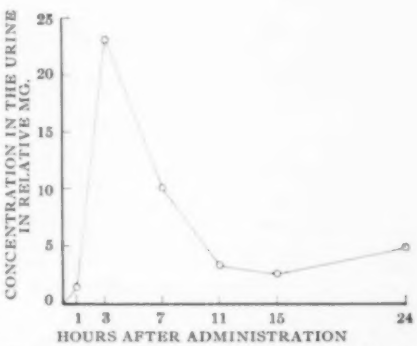

Fig. 2.—Excretion rate of the combination of sulfacetamide, methenamine, and mandelic acid in relative number of mg. This curve represents the antibacterial activity of the drugs as was determined by microbiological assay. Calculations were based on the assumption that the drugs were present in the urine in the same ratio as they were when administered. Only the quantities of sulfacetamide were plotted and the values of methenamine and mandelic acid may be assumed to be present in the proportions indicated on Table I.

DISCUSSION

Results of Attempts to Produce Resistant Strains.—The experimental results indicate that with one exception all the organisms studied are capable of developing some degree of resistance to all the drugs and drug combinations. The drugs were evaluated by means of the resistance and efficiency indexes found in Tables IV and V. Although strains resistant to sulfacetamide were produced with relative ease, progressive difficulty was encountered using the medium containing methenamine and mandelic acid. By combining methenamine and mandelic acid with sulfacetamide the mean resistance index was substantially increased. The combination of three drugs was demonstrated to possess a mean resistance index over 52% greater than the second ranking combination, sulfacetamide and mandelic acid. This combination was also shown to possess a higher mean efficiency index than the other combinations. The combination of three drugs received the highest resistance index for *Aerobacter aerogenes* and *Streptococcus pyogenes* while it displayed the highest efficiency index for *Aerobacter aerogenes*, *Micrococcus pyogenes* var. *aureus*, and

Streptococcus pyogenes. This combination ranked in the second position for *E. coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* by both systems of analysis. The combination of all three drugs apparently retains the advantageous qualities of each drug. It was shown that this combination possesses both the highest mean resistance index and the highest mean efficiency index. A study of the mean efficiency and resistance indexes of other combinations shows more varied results. Combination No. I (sulfacetamide and methenamine) was shown to possess the second highest mean efficiency index while it possesses the poorest mean resistance index. Also, combination No. II (sulfacetamide and mandelic acid) could be classified as poorest by the efficiency index system, while it was found to be second best by the resistance index system.

Discussion and Conclusions of the Excretion Studies.—Quantitatively varied results were obtained; however, it may be seen from Table VI and Fig. 1 and 2 that the maximum concentrations of the drugs appeared in the urine three hours after their oral administration. The antibacterial action of the drugs became apparent after one hour and persisted fifteen hours after administration. Little or no antibacterial action was observed after twenty-four hours. Carroll and Allen (13) have reported that the combination of methenamine and mandelic acid produces a sufficiently acid reaction in the urine. The results of this experiment show that the presence of sulfacetamide does not interfere with this action.

SUMMARY

1. Attempts were made to produce strains of six urinary tract pathogens resistant to sulfacetamide, methenamine, mandelic acid, and four combinations of these drugs.

2. The drugs were evaluated by means of their resistance and efficiency indexes. Utilizing these methods of analyses the combination of all three drugs in the proportions used was shown to be superior against most of the organisms tested.

3. The administration of all three drugs in combination to human subjects furnished evidence that the combination produced an acid urine which allows the maximum antibacterial action to be obtained from methenamine and mandelic acid.

Antibacterial concentrations were demonstrated to be present in the urine one hour after the administration of the combination of three drugs. Maximal concentrations were found after three hours and little or no effect was exerted twenty-four hours after administration.

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The Use of Hydrogenated Cottonseed Oil and Hexadienol as Suppository Bases*

By CHARLES W. HARTMAN† and JOSEPH P. LaROCCA

Suppository bases were formulated from mixtures of hydrogenated cottonseed oil and hexadienol with various other ingredients. Suppositories were prepared from these bases that were satisfactory with respect to appearance and consistency. The potential of the bases to release a medicament was determined by a colorimetric method. The effect of Veegum® and other agents on the release of medicament from the base is reported. Suppositories made from these bases were stored for one year and the results of storage are reported. A more detailed study of these bases using other emulsifying agents is recommended.

MUCH OF THE reluctance of the medical profession to use the suppository form of medication can be ascribed to the unreliability of obtaining a good product with respect to release of the medicament, proper melting point, and good storage quality. In this report an attempt has been made to formulate satisfactory suppository bases from hydrogenated cottonseed oil and hexadienol.

Gayatonde (1) and Waxman and Eiler (2) have studied the effect of various emulsifying

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TABLE I.—PER CENT COMPOSITION OF SUPPOSITORY BASES

Constituent	Suppository Base Number																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Cotmar®	90	70	..	90	..	..	..	..	..	..	..	..	..	..	..	..	..
Hexadienol	..	..	..	..	93	75	70	90	80	70	75	..	..	..	..	..	..
Hexene-ol®	..	..	..	..	..	..	..	..	..	..	..	90	93	90	85	80	80
Cotoflakes®	10	20	33 3	..	7	..	..	3	5	..	..	10	7	..	..	..	..
Powd. Veegum®	..	5	33 3	..	..	..	5	3	5	10	10	..	..	10	10	10	15
Stearic acid	..	..	..	7	..	..	..	3	..	7	..	..	..	..	5	10	5
Triethanolamine	..	..	..	3	..	..	..	1	..	3	..	..	..	..	..	..	..
Anhyd. lanolin	..	5	33 3	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cholesterol	..	..	..	..	..	..	..	..	5	..	5	..	..	..	..	..	..
Gly. monostearate	..	..	..	..	..	25	25	..	..	10	10	..	..	..	..	..	..
Dist. water	..	..	..	..	..	..	..	..	5	..	..	..	..	..	..	..	..
Storage quality[a]	G	E	G	G	E	E	F	G	E	F	P	F	G	F	P	P	P

[a] Appearance of suppositories after storage of one year: E, excellent; G, good; F, fair; P, poor.

agents in suppository bases and concluded that emulsifying agents are beneficial in fat-type bases. Bases similar to cocoa butter have been prepared by the selective hydrogenation of cottonseed oil (3) and from hydrogenated peanut oil (4). Caldwell (5) has used mixtures of hydrogenated palm kernel and hydrogenated soybean oil as a substitute for cocoa butter. Gross and Becker (6) have suggested many new bases and have given a comprehensive review of the literature on suppositories.

Although previous work has been done on the use of hydrogenated cottonseed oil as a suppository base, it was thought that mixtures of completely hydrogenated cottonseed oil and partially hydrogenated cottonseed oil would make a good base for suppositories used for nonsystemic therapy. Cotoflakes®[1] is completely hydrogenated cottonseed oil. It has a melting range of 58–62° and an iodine number of approximately 7. This was mixed in varying percentages with Cotmar®[2] which is partially hydrogenated cottonseed oil having a melting range of 35–39° and an iodine number of approximately 70. The melting points of the various mixtures are shown in Fig. 1. One might choose from the graph a mixture with any desired melting point. Various agents were added to these mixtures either to facilitate the release of medicament or to increase the stability of the suppository base. Acceptable formulas for suppository bases involving the use of hydrogenated cottonseed oil are given in Table I.

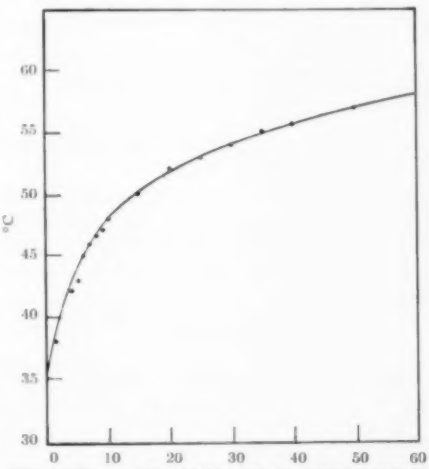

Fig. 1.—Melting points of mixtures of completely hydrogenated cottonseed oil and partially hydrogenated cottonseed oil.

Hexadienol as a Suppository Base.—Hexadienol[3] is a semisolid substance having the tentative general formula $C_nH_{2n-3}OH$ (7). The crude commercial product has a melting point of approximately 35°. It has a mild hydrophillic property but is insoluble in water (8). It is miscible with oils resulting in a strong affinity and marked reduction in surface tension. Physiologically it acts as a sudorific agent, has a mild analgesic effect and is generally considered to be nontoxic (9). With these properties in mind it was thought that hexadienol would make an excellent suppository base of the fat type for local therapy. Suppositories were prepared using hexadienol as the base in combination with hydrogenated cottonseed oil and other agents to increase the stability or to increase the potential for drug release. Suppositories were also prepared from Hexene-ol[4]. Hexene-ol® is an emulsion of hexadienol 70% and hydrous lanolin U. S. P. in water with small amounts of triethanolamine and glyceryl monostearate as stabilizing agents. Formulas for suppository

[1] Cotoflakes® and [2] Cotmar® were furnished by the Procter and Gamble Company, Cincinnati 17, Ohio.

[3] Hexadienol was furnished by the Hexene-ol Laboratory, Inc., Cleveland 15, Ohio.

[4] Hexene-ol® was furnished by the Hexene-ol Laboratory, Inc., Cleveland 15, Ohio.

TABLE II.—MELTING RANGES AND PER CENT OF DYE RELEASED FROM SUPPOSITORY BASES

Base No.	M.R.	Time in Minutes 1	2	3	4	5	8	11
1	45–47	0	0	0	0	0	0	0
2	49–51	0	0	0	0	0	0	1.0
3	54–56	0	1.7	1.7	2.5	2.5	11.4	14.0
4	37–40	4.7	4.7	6.2	6.2	7.2	8.7	10.1
5	43–45	0	0	0	0	0	0	0
6	41–43	0	0	0	0	0	0	1.5
7	42–46	0	0	1.3	2.0	2.0	2.7	5.2
8	49–51	0	0	0	0	0	0	0
9	42–44	0	0	0	0	0	0	0
10	41–44	0	0	1.8	1.8	2.6	3.5	5.2
11	40–44	0	0	0	0	0	2.8	14.4
12	47–49	1.0	2.9	2.9	4.8	5.7	6.6	9.1
13	44–46	0	2.5	3.3	3.3	4.9	6.4	6.4
14	41–44	0	0	1.3	1.3	1.9	2.6	5.1
15	47–49	1.5	1.5	2.3	2.3	2.3	3.0	5.9
16	48–50	1.7	1.7	1.8	2.0	3.3	6.9	9.6
17	56–58	0	0	0	1.8	2.8	5.5	12.1
18[a]	34–38	0	0	0	0	0	0	0
19[b]	36–40	1.5	2.9	3.0	4.3	4.3	6.7	8.5

[a] Hexadienol 100.0%. [b] Hexene-ol® 100.0%.

bases involving the use of hexadienol and Hexene-ol® are given in Table I.

Veegum[1] was used in some of the suppository bases to serve as a disintegrator and to improve stability. The Veegum® was powdered in a ball mill so that it would pass through an 80-mesh sieve.

EXPERIMENTAL

Preparation of Suppositories.—All suppositories were prepared by fusion of the ingredients at the lowest possible temperature. A mold for rectal suppositories was used and no lubricant was used for the molding of any suppositories. After the suppositories were molded they were placed in a refrigerator (2–15°) for about two weeks. They were then left at room temperature ranging from 23 to 33° for at least one week and observed occasionally to determine their storage qualities. Only those suppositories that had a good appearance after a week were studied in this work. The melting range of all suppositories was determined by the U. S. P. XIV method for Class II materials (10).

Colorimetric Test for Release of Dye.—The colorimeter method for testing the potential for drug release of the bases is the one proposed by Gross (6) with slight modifications. Amaranth F. D. and C. Red No. 2, was used as the standard to test the release of a drug from the suppository bases proposed. The dye was incorporated in an approximately equal amount of the base without the aid of water or other solvents. Then the rest of the base, which consisted of two previously molded suppositories, was added by melting and stirring the mixture. From the resultant mixture one suppository containing dye was molded. One suppository containing dye and one blank suppository were placed in the two percolators as described by Gross and Becker (6). The percolators contained 400 cc. of distilled water maintained at approximate body temperature with a constant temperature bath. At definite time intervals 5-cc. samples were withdrawn from the percolators with volumetric pipets and placed in 100-cc. volumetric flasks which were brought to volume with distilled water. The concentration of dye in these solutions was determined with a Coleman Jr. spectrophotometer. The wavelength of absorption was set at 525 mμ. Results of the colorimetric tests are given in Table II.

Storage of Suppositories.—After the tests had been completed on the suppositories the remaining suppositories were placed in suppository boxes without being wrapped in any protective paper and stored at room temperature for at least one year. The room temperature over this period of time varied between 0 and 40°. Those bases which were still considered satisfactory after one year storage are indicated in Table I.

DISCUSSION AND RESULTS

Table II gives the results obtained from tests conducted on the suppository bases proposed in this paper. Melting points were taken on several proprietary suppositories from different companies containing fat-type bases with other added ingredients. The melting points of all of these bases were between 48 and 60°. This would indicate that a base must have a high melting point in order to have an acceptable shelf life. Suppositories with a high melting point may be prepared from hexadienol and completely hydrogenated cottonseed oil. The plastic nature of hexadienol gives the base a soft appearance but at the same time it makes the base more stable because it will not become brittle. Suppositories prepared by fusion using hexadienol have an excellent appearance. Suppositories made from hydrogenated cottonseed oil are more stable and have more pharmaceutical elegance than other fat-type bases now being used.

Veegum® was used in some of the suppository bases. As can be noted from Table I it increased the amount of dye released by the suppository. It is believed that its action was partially masked in these experiments because of the adsorption affinity of Veegum® for the dye.

[1] The Veegum® was furnished by the R. T. Vanderbilt Co., New York, 17.

It would seem from the data that there is no great advantage in the use of emulsifying agents in high-melting fat-type bases. The authors are now in the process of making a more detailed study of these bases using some of the newer emulsifying agents.

SUMMARY AND CONCLUSIONS

1. Mixtures of completely hydrogenated cottonseed oil and partially hydrogenated cottonseed oil are proposed as fat-type suppository bases. Bases made from these mixtures are seemingly superior to those formulated from other fat-type bases now being used.

2. Hexadienol and Hexene-ol®, an emulsion of hexadienol, are suggested for use as suppository bases. These bases appear to have advantages over many of the bases now being used and should be studied in greater detail.

3. The effect of certain emulsifying agents on the potential for the release of a drug has been studied. These agents do not greatly affect the rate of release from fat-type bases, with melting points higher than body temperature, as shown by the results of the colorimetric method. Since this is in conflict with previous reports, further and more detailed studies should be made with these and other emulsifying agents.

4. The use of Veegum® as an aid in suppository disintegration has been investigated. It shows promise and further studies on its use in suppositories should be made.

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A Preliminary Study of the Pharmacology of 11-Desmethoxyreserpine[1] (Raunormine®)[2] an Alkaloid from *Rauwolfia canescens* II*

By ELIAS W. PACKMAN, DONALD D. ABBOTT, and JOS. W. E. HARRISSON

11-Desmethoxyreserpine (*alpha* desmethoxyreserpine) a new ester alkaloid isolated from *Rauwolfia canescens*, was examined for its pharmacologic effects upon rodents and dogs. This alkaloid possesses hypotensive and tranquilizing effects comparable to reserpine. In addition, it produces hypothermia, increased salivation, miosis, and increased gastric acid secretion.

FOR SOME YEARS the root of *Rauwolfia serpentina*, a plant indigenous to India, has been used in this country in the treatment of hypertension (1). However, it was not until 1952 that Muller, Schlittler, and Bein (2) isolated from this root an alkaloid known as reserpine which possessed not only a hypotensive action, but also a calming or sedative effect.

Since this species of Rauwolfia is exported in rather limited amounts, the availability of reserpine is also limited. Therefore, when an alkaloid was isolated recently from *Rauwolfia canescens*, the structure of which is similar to that of reserpine, the authors determined to investigate the pharmacologic action of this alkaloid for which we have suggested the name *alpha* desmethoxyreserpine.

In a previous report Harrisson, *et al.* (3), presented some of the physical and chemical properties of *alpha* desmethoxyreserpine as well as data concerning the toxicity of this alkaloid in mice, rats, and dogs and have referred to the several concurrent studies by other investigators on the chemistry and pharmacology which have resulted in the alkaloid being termed: canescine, deserpidine, and recanescine. Employing these data as guides, the authors have attempted to evaluate the hypotensive and calming effect of this new alkaloid from *Rauwolfia canescens*.

* Received April 28, 1955, from LaWall and Harrisson Research Laboratories, Philadelphia 3, Pa.
Presented to the Scientific Section A.PH.A., Miami Beach meeting, May 1955.
[1] *alpha* Desmethoxyreserpine.
[2] Raunormine is the registered trademark of S. B. Penick & Company for their 11-desmethoxyreserpine which was used in this study.

METHODS

Cardiovascular Effects.—Mongrel dogs weighing 8–12 Kg. were anesthetized by intravenous injection of 25 mg./Kg. sodium pentobarbital in normal saline solution. Mean arterial blood pressure was obtained by canulating the femoral artery with a polyethylene cannula and recording the pressure directly from a mercury manometer. The entire system leading from the artery to the manometer was constructed of polyethylene tubing filled with a solution containing 12.5% magnesium sulfate and 7% sodium sulfate.

To observe the effect on the carotid sinus pressor reflex, the following procedures were employed. A midline incision was made in the neck region and the two common carotid arteries exposed. The vagus nerve was carefully separated from the arterial sheath and a loose ligature placed around the carotids so that they were easily accessible. Occlusion of the carotids was accomplished by clamping the vessels with a rubber-covered hemostat for fifteen seconds; this produced a rise in pressure, on a normal dog, of approximately 25 mm. of mercury.

Injections were made intravenously by way of the femoral vein of the leg opposite from the one used to record blood pressure.

In general, mean arterial pressure was observed for a minimum of two hours following intravenous administration, while the effect upon carotid occlusion was observed at fifteen-minute intervals during the same period.

Two groups of control animals were employed in this experiment: One group received the solvent intravenously, while the second group received only the pentobarbital anesthetic. Blood pressure of dogs in these groups was determined by the same procedure employed in the experimental groups.

Sedative and Tranquilizing Effect.—Eighty male Webster, Swiss, albino mice weighing 18 to 23 Gm. were divided into four groups, each containing 20 animals. The following substances prepared in dilute acetic acid were administered orally: Group 1, 0.6 mg./Kg. reserpine, Group 2, 1.2 mg./Kg. reserpine, Group 3, 0.6 mg./Kg. 11-desmethoxyreserpine, Group 4, 1.2 mg./Kg. 11-desmethoxyreserpine.

Evaluation of the "ptosis response" was made as described by Rubin and Burke (4). 0 = no change, 1 = 1/4 reduction in vertical distance between eyelid margins, 2 = 1/2 reduction, 3 = 3/4 reduction, 4 = complete closure of eyelids. The response for each mouse was the sum of the scores of both eyes.

To study the tranquilizing effect three unanesthetized mongrel dogs weighing 9 to 12 Kg. were administered intravenously 1 mg./Kg. 11-desmethoxyreserpine and the effects observed over a 24-hour period.

Effects upon Gastric Secretion.—0.5 mg./Kg. 11-desmethoxyreserpine was injected intravenously to mongrel dogs prepared with simple Pavlov gastric fistulas. Gastric juice was sampled during an eight-hour period. Free and total acid was determined for each sample by titration with 0.01 *N* NaOH using bromophenol blue and phenol red as indicators.

Preparation of Solutions for Intravenous Injection.[3]—*11-Desmethoxyreserpine 2 mg./Kg. or reserpine 2 mg./Kg.*—A weighed quantity of alkaloid was dissolved in a 1:1:2 mixture of ethyl alcohol, propylene glycol, and water. The sample was first triturated with the alcohol and propylene glycol and dilute hydrochloric acid added dropwise until a clear solution was formed. The solution was then brought to volume with water.

The solutions were freshly prepared before each experiment and were adjusted so that the maximum volume injected intravenously did not exceed 5 ml.

Solvent.—Control animals receiving solvent intravenously were injected with 5 ml. of the ethyl alcohol, propylene glycol, and water mixture.

Preparation of Solutions for Oral Administration.—The solutions were prepared by dissolving 120 mg. of sample in 10 ml. of a 10% acetic acid solution and then stored under refrigeration. For assay 1 ml. of this stock solution was diluted to 100 ml. with distilled water so that the resulting concentration was 0.12 mg./ml.

RESULTS

Cardiovascular Effects.—Fifteen of sixteen dogs receiving 11-desmethoxyreserpine intravenously exhibited a hypotensive response. All of these animals showed a drop in blood pressure within a maximum time of two hours following injection. The maximum fall in arterial blood pressure was 100 mm. Hg (Fig. 1). The mean fall in pressure was

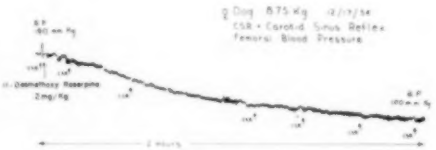

Figure 1.

36 mm. of mercury, the range being 4–100 mm. (Tables I and Ia). In one dog the blood pressure did not fall, but instead there was an 80-mm. rise during the first thirty minutes after injection. The pressure of this animal then dropped gradually and approached the initial figure in three hours. Bradycardia was produced in the anesthetized dogs, but no other E.K.G. changes were observed. Similar results were obtained in the unanesthetized dog. The bradycardia observed in the isolated frog heart preparation upon administration of 11-desmethoxyreserpine may be eliminated by atropinization.

Practically no decrease in arterial pressure was observed in control animals. Of four dogs receiving the alcohol-propylene glycol-water mixture the mean drop in pressure was 3 mm., while those dogs receiving no injection also showed a 3-mm. decrease in blood pressure (Tables I and Ia). No bradycardia was observed after administration of solvent to anesthetized or unanesthetized dogs. Similar negative findings were observed in the isolated frog heart preparation.

In eight of ten dogs, 11-desmethoxyreserpine abolished the reflex pressor responses following

[3] 11-Desmethoxyreserpine (Raunormine®) and reserpine were generously supplied by S. B. Penick & Company.

TABLE I

MEAN ARTERIAL BLOOD PRESSURE IN MM Hg

11-DESMETHOXY RESERPINE 2 MG/KG			SOLVENT CONTROL			RESERPINE 2 MG/KG		
INITIAL	END	MAXIMUM FALL	INITIAL	END	MAXIMUM FALL	INITIAL	END	MAXIMUM FALL
200	175	25	240	235	5	160	144	16
240	220	20	140	136	4	120	100	26
220	190	30	120	125	4	140	135	5
210	196	16	110	115	0			
220	220	4						
240	203	37	MAXIMUM FALL IN PRESSURE 5 MM			MAXIMUM FALL IN PRESSURE 26 MM		
235	172	63						
245	204	55						
190	100	90	RANGE 0 - 5 MM			RANGE 5 - 26 MM		
190	135	55						
190	136	54	MEAN FALL IN PRESSURE 3 MM			MEAN FALL IN PRESSURE 16 MM		
140	138	8						
140	120	28						
110	73	37						
110	90	20	CONTROL (NO INJECTION)					
			250	255	0			
MAXIMUM FALL IN PRESSURE 100 MM			160	165	10			
			120	120	0			
RANGE 4 - 100 MM								
			MAXIMUM FALL IN PRESSURE 10 MM					
MEAN FALL IN PRESSURE 36 MM								
			RANGE 0 - 10 MM					
ONE DOG EXPERIENCED A RISE IN PRESSURE								
220	240	+20	MEAN FALL IN PRESSURE 3 MM					

TABLE Ia

MEAN ARTERIAL BLOOD PRESSURE IN MM Hg

11-DESMETHOXY RESERPINE 2 MG/KG	SOLVENT CONTROL	CONTROL NO INJECTION	RESERPINE 2 MG/KG
MEAN FALL IN PRESSURE 36	3	3	16
RANGE 4-100	0-5	0-10	5-26
MAXIMUM FALL IN PRESSURE 100	5	10	26

carotid occlusion. Abolishment of this response occurred within two hours of injection. The reflex pressor response to carotid occlusion was again evident in two of the dogs two and three quarters hours after injection.

No visible effect on the reflex pressor response was noted in the two remaining dogs.

Sedative and Tranquilizing Effect.—The ptotic effect produced by 11-desmethoxyreserpine in mice is manifest at the same concentrations as reserpine; however, the degree of this response appears to be somewhat less. The individual ptotic scores of the mice exhibit the usual variation (Table II). 11-Desmethoxyreserpine exhibits ptotic activity of 71% when compared to reserpine with a potency range not differing materially from that encountered in reserpine assays (Tables III and IV). Hypothermia was observed in mice receiving 11-desmethoxyreserpine as well as those receiving reserpine.

TABLE II.—PTOTIC ACTIVITY

11-Desmethoxyreserpine (U) N = 20 Ptotic Scores			
0.6 mg./Kg.		1.2 mg./Kg.	
8	6	8	6
6	6	4	8
2	4	6	6
4	5	8	6
6	4	8	4
4	0	4	4
7	4	4	6
0	6	6	8
0	6	6	6
0	2	2	6
$T = 80(U_1)$		$T = 116(U_2)$	

Reserpine (S) N = 20 Ptotic Scores			
0.6 mg./Kg.		1.2 mg./Kg.	
8	6	8	7
4	4	8	6
6	4	7	5
0	5	8	8
5	8	6	7
6	7	6	8
0	0	6	8
6	6	8	6
6	5	8	6
4	5	8	8
$T = 95\ (S_1)$		$T = 142\ (S_2)$	

The tranquilizing effect produced by 1 mg./Kg. 11-desmethoxyreserpine was evident in dogs within forty to sixty minutes after a single intravenous injection. Interest was lost in the surroundings and they were noticeably quiet. At the same time the animals exhibited a gradual head drop (Fig. 2) which was followed by a buckling of the front legs. This

TABLE III.—11-DESMETHOXYRESERPINE

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Analysis of Variance				
Among Doses	3	108.6	36.2	
Preparations	1	...	D_2 = 21.01	
Regression	1	...	B_2 = 86.11	9.11
Parallelism	1	...	1.512	
Error	76	302.8	S_2 = 3.98	
Total	79	411.4		

Factorial Analysis

	S_1	S_2	U_1	U_2	Sum of Products	Divisor	Sum of Squares
Preparations	−1	−1	+1	+1	−41	80	21.01
Regression	−1	+1	−1	+1	+83	80	86.11
Parallelism	+1	−1	−1	+1	+11	80	1.512

Reserpine (S)
$log\ 0.6 = 9.77815–10$
$log\ 1.2 = 0.07918$
$X_s = 4.97866–5$

11-Desmethoxyreserpine (U)
$log\ 0.6 = 9.77815–10$
$log\ 1.2 = 0.07918$
$X_U = 4.97866–5$

TABLE IV.—POTENCY OF 11-DESMETHOXYRESERPINE

N = 1.00 (for a two-dose assay)
I = 0.301 = log 2.0
D = 4.58
B = 9.28

Log potency = $M = \bar{X}_s - \bar{X}_u + KID/B = 0.1486$
Antilog M = 0.71 = potency of 11-desmethoxyreserpine (U) relative to reserpine (S)

Log limits of error = $S_M = \dfrac{sKI\,(D_2 + B_2)}{B_2} = 0.072$

Log limits of error = $M \pm t(80)S_M$
At 95% confidence limits = $-0.1486 \pm 1.99\,(0.072)$
Antilog = 0.51 − 0.99

Potency of 11-desmethoxyreserpine = 71% of reserpine
Potency range 51–91%

Figure 2.

position was maintained with the hind portion of the body upright while the forelegs and head were completely relaxed (Fig. 2) for approximately ninety minutes, after which the animals lay down. However, they were readily aroused and responded in normal manner to touch and sound.

In addition to the tranquilizing effect the following observations were made in the dog: Miosis was evident almost immediately after injection and did not disappear throughout the twenty-four-hour observation period; an increase in salivation as well as dyspneic respiration was observed thirty minutes after injection; hypothermia was observed in the dogs two hours after injection. Similar results were obtained with reserpine in rabbits by Bein (5).

Defecation was greatly increased after injection and the stools were more liquid than usual, indicating a stimulation of peristalsis. In contrast to this observation we have found that 11-desmethoxyreserpine in a concentration of 0.01 mg./ml. relaxes the isolated guinea pig ileum. Plummer, *et al.* (6), have reported similar effects with reserpine upon the rabbit ileum.

Effect on Gastric Secretion.—Intravenous injection of 0.5 mg./Kg. 11-desmethoxyreserpine to dogs produced a large volume of gastric juice over an eight-hour period. In this species the volume and the free and total acidity of the gastric secretion were greater than that produced with comparable doses of histamine.

DISCUSSION

Cardiovascular Effects.—11-Desmethoxyreserpine when administered intravenously to the dog in a dosage of 2 mg./Kg. produces a fall in mean arterial pressure regardless of initial blood pressure of the animal. This reduction in blood pressure appears to be a steady but gradual decrease which reaches its lowest level in approximately two hours.

The fall in blood pressure produced may be due to a reduction in peripheral resistance which is associated with the depression of the reflex pressor response following carotid occlusion. This evidence originally presented by Trapold, Plummer, and

Yonkman (7) in regard to reserpine may pertain to 11-desmethoxyreserpine since the two alkaloids exert similar effects on the carotid sinus reflex.

These preliminary findings indicate that 11-desmethoxyreserpine is at least as effective in reducing blood pressure, after intravenous injection, as is reserpine.

It appears that the methoxy group at position 11 in the reserpine molecule is not necessary for its hypotensive effect. Since the fall in the mean arterial pressure occurs within two hours of injection it may be that the absence of the methoxy group at position 11 in desmethoxyreserpine actually is beneficial from the standpoint of onset of action and degree of hypotensive effect.

The dogs in which a relatively high initial arterial pressure was observed seemed to be in a hyperactive state and information from the supplier of these animals indicated that these particular dogs were "fighters." This increase in activity and the fact that the blood pressures were recorded through an all-polyethylene system, which is more rigid than rubber, may explain the initial pressure readings of 200 mm. and over, since the actual pressure is transmitted to the mercury. In our own laboratory we have found that the blood pressure may differ by as much as 20 mm. when the pressure is recorded, first through a rubber system and then through an all-polyethylene system.

Upon autopsy of these so called "hypertensive" dogs, no gross pathology was noted.

Sedative and Tranquilizing Effect.—Considering the ptosis test as a criterion of sedation, 11-desmethoxyreserpine has a sedative activity apparently slightly less than is produced with an equal dose of reserpine. The phenomenon of ptosis produced is probably due to decreased sympathetic predominance.

The quiescent state following intravenous injection of 11-desmethoxyreserpine to the dog is of a different character from that produced by barbiturates. Unlike the deep sedation following barbiturate administration, the dogs receiving desmethoxyreserpine exhibited no apparent mental confusion or difficulty of movement and they could readily be aroused by normal stimuli.

CONCLUSIONS

11-Desmethoxyreserpine (*alpha* desmethoxyreserpine) produces a condition characteristic of general parasympathetic predominance. The results obtained thus far seem to be in close agreement with those obtained by Muller, Schlittler, and Bein (2, 5) and by Plummer, Schneider, Trapold, and Barrett (6) on reserpine. Therefore, this new alkaloid isolated from *Rauwolfia canescens* may not only prove to be an able substitute for reserpine, but may also play an important role in determining which structures are necessary for the pharmacologic action of Rauwolfia alkaloids.

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Antibacterial Agents in *Pseudomonas aeruginosa* Contaminated Ophthalmic Solutions*

By S. RIEGELMAN, D. G. VAUGHAN, Jr., and MASAO OKUMOTO

The criteria of sterility of ophthalmic solutions has been based on *in vitro* bacteriological studies. *In vivo* rabbit corneal tests along with improved techniques of *in vitro* testing indicate that bactericidal concentrations of preservatives are required for maximum safety. Polymyxin B sulfate is the agent of choice.

SINCE 1953, the F.D.A. has considered a nonsterile ophthalmic solution as being adulterated and misbranded. Although in the legal sense, this regulation (1) affected only manufacturers and repackers, many studies have been reported since 1953 which have pointedly examined the problem from both the standpoint of the manufacturer and the retail pharmacist.

The selection of adequate chemical preservatives for ophthalmic solutions is by no means a simple procedure. The agent(s) must be effective against both Gram positive and Gram negative organisms with particular emphasis on *Pseudomononas aeruginosa*, since this organism poses the greatest hazard in ophthalmology. *In vitro* bacteriological studies which attempt to prove the sterilizing potentialities of an antibacterial agent obviously must be conducted by a method which assures the elimination of the residual bacteriostatic concentrations remaining in the

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culture medium. McPherson and Wood (2), Skolaut (3), Scigliano and Skolaut (4), Krause, Dauer, and Guth (5), Heller, *et al.* (6), and Murphy and co-workers (7) have all recently reported on the relative effectiveness of preservatives in ophthalmic solutions. Apparently none of these authors have reported the precautions which they took to assure the absence of bacteriostatic concentrations in their subcultures.

In testing of antibacterial action it is obvious that in order to kill bacteria by contact with an antibacterial agent, the bacteria must be in the presence of sufficient concentration for sufficient time interval for irreversible changes, producing a lethal effect, to take place within the bacterial cell. It is also true that bacterial death under a particular set of experimental conditions may depend largely upon the criteria applied. Frequently a variation in these conditions will reveal a complete revival of a presumably "dead" bacterial cell or at least a restoration of some of its biological functions. It is essential to ascertain the condition of the bacteria, following their exposure to the action of a given antibacterial agent, in order to learn whether or not the organisms, which, in the course of a given testing procedure, seemingly have been "killed" or otherwise rendered harmless, have actually ceased to be a source of infection when placed under more favorable conditions, such as might be found in the abraided cornea.

Klarmann (8) has presented the general picture of the role of chemical antagonists in the evaluation of antiseptics, while Lawrence (9) reviewed the various chemical antagonists of the action of quaternary ammonium chlorides. Bliss and Warth (10) have reported on the use of soap and of lecithin as antagonists for polymyxin B sulfate and other antibiotics.

At first glance the testing for bactericidal activity of benzalkonium chloride and phenylmercuric nitrate appears to be a simple procedure, since several recognized chemical antagonists are known for each compound. Klarmann discussed the use of thiol derivatives as chemical antagonists for the action of organic mercurials. The thioglycollate medium has had such general acceptance that it has become the standard medium for the testing of the sterility of biological materials. However, the work reported in this paper appears to indicate that thioglycollate medium, by itself, will not neutralize the action of phenylmercuric nitrate on the particular strains of *Pseudomonas aeruginosa* tested. Difficulties in neutralizing the action of benzalkonium chloride with naphuride sodium or with anionic surfactants were also encountered.

Test procedures are even more difficult to establish when attempting to evaluate the bactericidal activity of chlorobutanol and phenylethyl alcohol, since we do not possess chemical antagonists and must rely on dilution to deplete the bacteriostatic activity of the agent. Undoubtedly the diluting procedure will reduce the concentration of the unabsorbed chemical below the bacteriostatic concentration. However, dilution may have little or no effect on that portion which has become affixed to the bacterial cells and has initiated there a process that would lead ultimately to the destruction of their infectiousness unless interrupted. Even if dilution is an effective means of reducing the concentration of an antibacterial agent below the bacteriostatic concentration, the procedure is not without danger. Each dilution also reduces the number of viable organisms. Sufficient dilution to eliminate the bacteriostatic effect of the preservative might possibly "dilute out" the bacteria.

Since *Pseudomonas aeruginosa* is known to produce a fulminating type of infection, the possibility of establishing an *in vivo* test method utilizing rabbit corneas, appeared to have several potential advantages. Such a procedure could serve as a means of further evaluation of solution sterility. While the ideal *in vitro* test might detect a single living bacterium, an *in vitro* test procedure utilizing a poor chemical antagonist might be falsely interpreted as being sterile. By paralleling the rabbit test with a bactericidal test procedure, a clarification of the sterilizing potentialities might be achieved. The rabbit test could contribute information on the relative activity of the antibacterial agents and could afford a method of determining whether bacteriostatic concentrations are adequate safeguards against potential ocular infections.

EXPERIMENTAL

Materials.—Solutions of phenylmercuric nitrate up to a concentration of 1:10,000 were tested. Tests on a more concentrated solution would be meaningless, as the presence of halide ions in most ophthalmic solutions would result in a precipitation of the insoluble phenylmercuric halide.

Benzalkonium chloride[1] in concentrations up to 1:5000 was tested. This limit was chosen as higher concentrations are known to irritate the cornea.

Chlorobutanol in concentration up to a saturated aqueous solution (approximately 0.8%) were tested. The majority of studies centered on a 0.5% solution since higher concentrations were found to be too irritating.

Phenylethyl alcohol was tested up to a 2.0% solution. Tests were primarily at the 0.5% level since greater concentrations caused local irritation.

[1] Zephiran, Winthrop Laboratories, Inc., was used.

Polymyxin B sulfate in 50, 100, 500, and 1,000 u./cc. was studied.

General Bacterial Test Procedure.—A virulent *Pseudomonas aeruginosa* strain isolated from a human ocular infection was the main test organism of the study. A 1-cc. quantity of a twenty-four- or forty-eight-hour broth culture of the *Ps. aer.* (approximately 10^9 bacteria) was added to 9 cc. of sterile saline solution of the antibacterial agent being tested. In order to evaluate the effect of the bacterial population, dilutions of the original culture were made to counts as low as 100 bacteria per cc. The bacteria were exposed for periods of time varying from five minutes to one week. Different neutralizing procedures were attempted to destroy the action of the antibacterial agent without reducing the viability of the organisms. These included simple dilution through broth as well as the various chemical antagonists reported by Lawrence (9) and Klarmann (8) and those discussed above.

***In Vivo* Procedures.**—Approximately 0.01 cc. of the *Pseudomonas* contaminated antibacterial solution was injected at various time intervals intracorneally into two or more rabbit eyes. Studies of the effect of dilution of the original contaminated solution with sterile broth or with the inactivating agent were also undertaken. In one experiment chorioallantoic chick membranes were inoculated along with the broth and rabbit corneas. All animals and cultures were observed for one week. Corneal swabs were taken of the infected animals in order to recover and identify organisms from the infected area. Over 300 rabbits' eyes were utilized in the test series.

***In Vitro* Procedures.**—A number of different media and procedures were investigated. These included nutrient broth, serum fortified nutrient broth, thioglycollate broth, Avery's broth, various solid media, and finally, what has proved to be most effective, thioglycollate lecithin-polysorbate 80 broth. This medium is essentially that described by Armbruster and Ridenour (11) with the exception that polysorbate 80 (12) was used in place of polysorbate 20. The composition of the medium is as follows:

Thioglycollate fluid medium	29.5	Gm.
Polysorbate 80	30.0	ml.
Asolectin[1]	2.0	Gm.
Agar	5.0	Gm.
Distilled water to make *p*H 7.0	1,000	ml.

In certain polymyxin studies, heart infusion agar with 0.5% Asolectin was used as the culturing medium. The lecithin was dispersed in 4 cc. of glycerol with the aid of a mortar and then diluted with the remaining medium to 100 cc. Varying amounts of the *Pseudomonas*-contaminated antibacterial solution up to 1 cc. were added to 9 cc. of the culture medium. Tenfold serial dilution was done through at least four tubes.

The following procedure of evaluating bacteriostasis was adopted: One cc. of a sterile solution of the preservative being tested was serially (1/9) diluted through three or more sterile broth tubes. The twenty-four- or forty-eight-hour *Pseudomonas* culture was diluted to the range of 10^5 to 10^3 bacteria per cc. These were added to a set of the preservative-containing broth tubes. Three sets were prepared at varying levels of bacterial count.

[1] Asolectin, Associated Concentrates, Inc., Atlanta, Ga.

This procedure was done on the assumption that the dilution of the preservative into the broth tube prior to contact with the bacteria will eliminate bactericidal activity of the agent. The bacteria will, therefore, be added to a bacteriostatic or inactive concentration of the preservative. If the culture medium is adequate in neutralizing the bacteriostatic action, the bacteria will multiply.

RESULTS

A few control experiments were done to ascertain the number of bacteria required to produce an intracorneal infection. A dilution of the original twenty-four-hour culture to a population of approximately 10^4 bacteria per cc. still produced an infection. The quantity of solution injected (approximately 0.01 cc.) would contain in the order of 100 bacteria.

When the *Ps. aeruginosa* bacteria are brought in contact with the test preservative a certain per cent of the original population are killed during the test period.

Obviously, infections resulted only when an adequate number of bacteria remained in the one-hundredth of a cc. of inoculated solution. Several tests were made using heavy contamination such as was produced by dispersing the bacteria found in one or two colonies removed from a bacteria plate. The bacteria in such a colony are difficult to disperse and it was felt that some of the positive results may have been due to the presence of clumps of bacteria which actually may not have been exposed to the preservative.

The addition of 1 cc. of a twenty-four-hour culture to 9 cc. of preservative solution resulted in a bacterial population in the range of 10^7 to 10^8 bacteria per cc. at the moment of inoculation. In certain cases a thousandfold dilution at the end of the test period did not dilute the bacteria below the critical number required to produce an infection. This was the case with phenylmercuric nitrate and at least one time when it was attempted with benzalkonium chloride.

A consistent finding during this study was the fact that the organisms would produce infections and could be recovered from the cornea while the *in vitro* cultures were often negative.

In vitro subcultures into thioglycollate broth of phenylmercuric nitrate up to 1:10,000 concentration appeared to indicate that sterility was produced at the end of one hour exposure. However, corneal ulcers resulted even after one week exposure. The use of the lecithin-polysorbate 80-thioglycollate medium (11) eliminated this discrepancy between the *in vitro* and *in vivo* results. Insufficient tests were made to differentiate between the action of a 1:10,000 and a 1:25,000 concentration of phenylmercuric nitrate. It was noted, however, that the latter usually resulted in a more fulminating infection.

Benzalkonium chloride at 1:5,000 concentration seemingly sterilized a contaminated solution in less than five minutes when tested in nutrient broth. Using naphuride sodium or sodium lauryl sulfate, indefinite end points were obtained with some positive cultures up to one hour. The injection of sterile benzalkonium chloride, 1:10,000, into the rabbit's cornea produces iritis and a mild conjunctivitis and it was necessary to differentiate this chemical kera-

titis and conjunctivitis from a true infection. The intracorneal injections of the *Ps. aeruginosa*-contaminated 1:10,000 benzalkonium chloride solution resulted in positive infections in one attempt at one week exposure and in another instance at three days' exposure. A 1:5,000 benzalkonium chloride solution produced infections repeatedly after twenty-four hours' exposure. Although infections were produced when the contaminated benzalkonium chloride solution was injected without neutralization, a higher number and more fulminating infection was produced if the solution were first neutralized by a 1:9 dilution into sodium suramin (1%) or into the lecithin-polysorbate 80-thioglycollate broth. Polysorbate 80-thioglycollate medium resulted in concordant *in vitro* results. Transfer of the solution to chick chorioallantoic membranes also led to positive cultures. In one of our test procedures a 1:5,000 benzalkonium chloride solution was inoculated with 1 cc. of a twenty-four-hour culture of the same strain of *Ps. aeruginosa* as in most of our previous tests. In this instance, however, the benzalkonium chloride was found lacking not only in bactericidal activity as shown by the production of an infection after twenty-four hours' exposure, but the solution was also completely lacking in bacteriostatic activity. Even in the presence of unneutralized benzalkonium chloride, the bacteria began to multiply and produced the green pigment common to this species. Conferences with Drs. Jawetz and Gunnison of the University of California Medical School, Department of Microbiology confirmed the observation that certain strains of *Ps. aeruginosa* have been found to multiply in the presence of 1:5,000 or even higher concentrations of benzalkonium chloride.

Only a limited study of phenylethyl alcohol was undertaken because of its irritating nature, and its reported ineffectiveness against several strains of *Ps. aeruginosa* (7). Preliminary studies indicated that concentrations greater than 0.75% were too irritating to the eyes of the authors. Corneal ulcers were produced from intracorneal injections of a contaminated 2.0% phenylethyl alcohol solution after eight hours' exposure (the longest period studied).

Chlorobutanol at 0.5% concentration appeared to sterilize a contaminated solution at forty-five and sixty minutes contact if the subculture medium was nutrient broth, thioglycollate broth, or Avery's blood broth. The use of lecithin-polysorbate 80-thioglycollate broth resulted in an *in vitro* evaluation of bactericidal activity at the end of four hours. The *in vivo* intracorneal injection produced infections up to four hours consistently with occasional growth after six hours' exposure.

In the course of studying the effect of surface active agents on the antibacterial action of antibiotics, Bliss and Warth (10) noted that lecithin (Asolectin) was an effective chemical antagonist for polymyxin B sulfate against *E. coli*. However, when polysorbate 80 was combined with the lecithin, the combination failed to antagonize the activity of the polymyxin. Our preliminary studies confirmed this observation. The bacteriostatic test procedure indicated a minimum inhibitory concentration above 1 unit per cc. However, when contaminated solutions were tested at five-minute intervals in the same media, all the cultures were negative even through 0.001 unit per cc., and agar subcultures were also negative. When 0.5% lecithin (no polysorbate 80) in nutrient broth was used as the diluting medium, negative cultures and subcultures were also obtained. Ultimately it was found that the use of 0.5% lecithin in heart infusion agar was an adequate system to antagonize the carry-over activity of polymyxin B. One cc. of the contaminated solution was diluted with 9 cc. of sterile agar at five-minute intervals. Colonies were detected in the agar plates up to fifteen minutes' exposure. Intracorneal injections of these contaminated polymyxin solutions (1,000 u./cc.) with or without dilution resulted consistently in infections up to fifteen minutes' exposure. No infections resulted at thirty or sixty minutes' exposure. Concentrations of polymyxin B sulfate of less than 1,000 u./cc. did not sterilize the solution in thirty minutes. From the brief tests, it appeared that 500 units per cc. will produce a sterile solution in approximately one hour.

A summary of the bacteriological observations is given in Table I.

DISCUSSION

The results reported are in marked disagreement with those reported by other investigators. Had we conducted only *in vitro* studies our conclusion might

TABLE I.—BACTERIOLOGICAL TEST DATA

Antibacterial Agent	Concentration	Longest Exposure Producing Infections[a]	Sterilizing Time[b]	Recommended *in Vitro* Culture Media
Phenylmercuric nitrate	Up to 1/10,000	1 Week[c]	?	Lecithin-Tween® 80-thioglycollate broth
Benzalkonium chloride	Up to 1/5,000	1–3 Days	>3 days	Lecithin-Tween® 80-thioglycollate broth
Phenylethyl alcohol	0.5–2.0%	8 Hours[c]	?	?
Chlorobutanol	0.5% + 0.8%	4–6 Hours	8 (?) to 24 Hrs.	Lecithin-Tween® 80-thioglycollate broth
Polymyxin B sulfate	Up to 1,000 u./cc.	15 Minutes	30 Minutes	Lecithin (0.5%) in heart infusion agar

[a] The antibacterial agent was contaminated with a heavy inoculum of a *Pseudomonas aeruginosa* strain. After varying time periods, a portion of the solution was injected intracorneally into rabbits. Corneal ulcers developed if viable bacteria were present.

[b] Evaluated as the shortest time interval which resulted in negative *in vivo* and *in vitro* tests.

[c] Longest time period.

[d] *Pseudomonas aeruginosa* bacteria have been detected in 1:500 solution of benzalkonium chloride instrument sterilizing solutions in clinic use. After several weeks the bacteria were still detectable and on one occasion were multiplying.

have been more concordant with the earlier reports. It is apparent from our studies that the accepted activity of several of the compounds tested rests on inadequate test procedures.

One should not be deluded into the conclusion that the cornea, in the ultimate sense, is a more sensitive test environment than the test tube. The problem centers around finding an *in vitro* procedure to eliminate the effect of the antibacterial agent thereby allowing the real sensitivity of the *in vitro* method to be indicated. Most of the antibacterial agents tested in this series may be assumed to be avidly absorbed on the surface of the *Pseudomonas aeruginosa* bacteria. Simple dilution alone or combined with certain antagonists did not displace the antibacterial agent, extending the exposure period into the subculture.

On the other hand the cornea has been shown to be an excellent culture substrate for the growth of *Pseudomonas* organisms apparently eliminating the carry-over activity of all of the preservatives tested. Differing from most other human tissues (the kidneys excepted) the corneal tissue presents a cultural environment extremely rich in the required growth nutrients while markedly limited in normal body serum and cellular defense mechanisms.

Twelve to twenty-four hours after the inoculation of 0.01 cc. of a *Pseudomonas*-contaminated solution, a diffuse infection became apparent. The conjunctiva was red, the cornea hazy, and the iris blood vessels prominent. The process varied in the next few days in its progression. Some of the infections became fulminating, characterized by copious purulent discharge and corneal perforation. Others were slowly progressive. These differences were probably due to many factors, such as the type of antibacterial agent being tested, number of viable organisms, variation in the actual volume injected, and normal animal variation. Figure 1 indicates the progress of a typical infection.

Most commercial ophthalmic solutions contain bacteriostatic concentrations of the chemical preservatives studied in this series, or they contain members of the same chemical families. Apparently, their selection of the bacteriostatic concentration levels is based on the assumption that these concentrations create an adequate margin of safety prior to and during the use of the solution.

One may wonder whether such concentrations are adequate, since even after a week's contact with bacteriostatic concentrations of quaternary ammonium chloride and phenylmercuric nitrate we repeatedly obtained intracorneal infections. Our study has been restricted mainly to a few strains of *Pseudomonas aeruginosa* and much more work would be required before any gross procedural changes of manufacture were suggested. Nevertheless, the data at hand lend themselves to a conclusion that preservative concentrations bactericidal to *Pseudomonas aeruginosa* should be used in preparing ophthalmic solutions. Our results tend to suggest the use of polymyxin B (combined with a Gram-positive bactericide), and under certain restrictions, chlorobutanol. A suggested method for the application of these results to the compounding of ophthalmic solutions will be published elsewhere (13). NOTE: Upon completion of this and prior to publication, the additional *in vitro* studies of Lawrence (14) were published. Since he has studied many of the same antibacterial agents as reported in this study, it is interesting to note his conclusion that benzalkonium chloride was the preservative of choice for ophthalmic solutions. Several of our observations are in contradiction to this conclusion. As stated above, we have been able to obtain infections in several strains after twenty-four or more hours' exposure to a 1:5,000 concentration of benzalkonium chloride.

We also wish to emphasize that *Pseudomonas aeruginosa* has been found to multiply actively in 1:5,000 benzalkonium chloride and even in a 1:500 benzalkonium chloride cold sterilizing solution. In several of these instances there was no possibility of any additive having neutralized the action of the benzalkonium chloride. This matter is under further study to attempt to clarify the disparity of results.

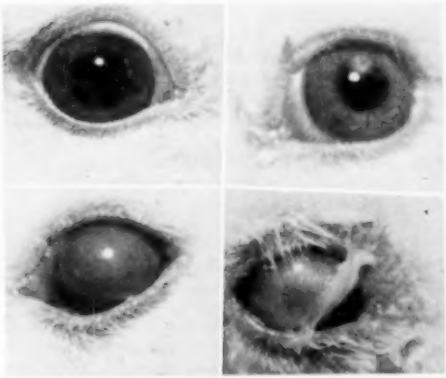

Fig. 1.—Stages in experimental Pseudomonal ulcer. *Upper left:* normal rabbit eye. *Upper right:* eighteen hours after intracorneal injection of Pseudomonas organisms. *Lower left:* two days after injection. *Lower right:* three to four days after injection. (Different rabbits were chosen for illustrative purposes.)

SUMMARY

1. The results are reported on *in vivo* (rabbit eyes) and *in vitro* bacteriological experiments.

2. Discrepancies between the earlier published *in vitro* data and this report are postulated to be due to an inadequate neutralization of the antibacterial agent in the culturing procedures.

3. Polymyxin B sulfate (1,000 u./cc.) is shown to be the agent of choice as a bactericidal preservative for *Pseudomonas aeruginosa*.

4. Bacteriostatic concentrations are not an adequate level of preservation for ophthalmic solutions.

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A Comparison of Methods of Assay of Digitoxin Tablets*

By W. A. MANNELL, A. LAVALLEE, RITA A. CARIOTO, and M. G. ALLMARK

A comparative study was made of the assay of digitoxin tablets by the official Canadian method (essentially the same as the U. S. P. XIV pigeon method) and by a preliminary draft of the U. S. P. XV method. A slight modification of the latter procedure was found to give more accurate results for digitoxin content and was incorporated in the U. S. P. XV method of assay. Sixty per cent of the representative samples taken of preparations on sale in Canada failed to meet the requirements of U. S. P. XV because of low digitoxin content. Only 11 per cent of the samples failed to comply with present Canadian requirements. It is apparent that a greater purity of product will be required of some manufacturers to comply with the U. S. P. XV monograph on digitoxin and digitoxin preparations.

THE U. S. P. XV has adopted a new method of assay for digitoxin and preparations containing digitoxin. In this method, digitoxin is first isolated by differential elution from a chromatographic column and then estimated colorimetrically using the alkaline picrate reagent.

This method supersedes the method in U. S. P. XIV which in turn replaced the biological assay of U. S. P. XIII. Braun and Lusky (1), in a comparative study, found that 25 per cent of the digitoxin samples examined gave results which were more than 25 per cent higher by the U. S. P. XIV chemical method than by a biological assay using pigeons. Other workers (2, 3, 4) have also observed lack of agreement in results obtained by chemical and biological methods.

Since the U. S. P. XIV assay for digitoxin was never adopted as an official method in Canada it appeared desirable to investigate the U. S. P. XV method as a possible replacement for the biological method of assay (which is essentially the same as that described in U. S. P. XIV for digitalis tincture). To this end a series of 18 samples of digitoxin tablets from 13 different manufacturers were assayed by both methods and the results were compared.

EXPERIMENTAL

The procedures used were the pigeon method described in U. S. P. XIV (5) and a preliminary draft of the U. S. P. XV method for digitoxin tablets, which was essentially the procedure described by Banes (6). In brief the latter method consists of dissolving the digitalis glycosides in formamide, mixing with a diatomaceous silica to form the immobile phase of a chromatographic column and then separating the digitoxin quantitatively by eluting with 190 ml. of benzene-chloroform (3 + 1) solution. The colorimetric determination of digitoxin, using the alkaline picrate reagent, is carried out on an aliquot of the eluate, the digitoxin content of the sample being determined by comparison with a solution of U. S. P. digitoxin reference standard.

In the present investigation a second eluate, consisting of 100 ml. of chloroform, was also routinely collected and assayed by the same colorimetric method. In addition to the quantitative analysis the two eluates were subjected to paper chromatography, using the method described by Silberman and Thorp (7), to determine the glycosides that were present and their approximate relative proportions.

In the first experiments using the chemical procedure we were impressed by the amount of digitoxosides found in the second eluate after the initial elution with 190 ml. of benzene-chloroform solution. Qualitative analysis by paper chromatography revealed the presence of digitoxin in the second eluate of 8 out of 15 samples of digitoxin tablets. The amount present varied from a trace to a considerable percentage of the total glycoside content of the second eluate. The second eluate also contained what was assumed from chromatographic evidence to be gitoxin in 14 of the 15 samples and a third glycoside was present, in small amounts, in 8 of the samples.

It was apparent that 190 ml. of benzene-chloroform did not consistently remove all the digitoxin from the chromatographic column. To investigate this discrepancy a column was prepared containing a known quantity of U. S. P. digitoxin reference

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standard. It was eluted with a total of 320 ml. of the benzene-chloroform solution, the eluate being collected in 8 fractions of 40 ml. each. The second eluate, 100 ml. of chloroform, was then passed through the column and was collected in 5 fractions of 20 ml. each. The 13 fractions were assayed quantitatively by the alkaline picrate reaction and qualitatively by paper chromatography for digitoxosides. The results are summarized in Table I. It is obvious that the volume of benzene-chloroform prescribed in the preliminary version of the U. S. P. XV method (190 ml.) removed a very large percentage of the digitoxin from the column when U. S. P. digitoxin reference standard was used (97% in this experiment). However, it is equally apparent that, under the conditions of the experiment, traces of digitoxin were still being eluted by the first two fractions of the chloroform eluate. It should be explained that there is about a 30-ml. liquid holdup in these columns which means that all of the first and part of the second fraction of the chloroform eluate is in fact benzene-chloroform. The first fraction collected, which contained no digitoxin, is chiefly isooctane left in the column after a preliminary wash. The gitoxin which was present in the U. S. P. digitoxin reference standard was eluted in the third and fourth chloroform fraction.

Table I.—Recovery of U. S. P. Digitoxin Reference Standard from Chromatographic Column

Fraction	Cumulative Volume (ml.)	% Recovery	Cumulative % Recovery	Glycosides Present
1 (First eluate)	40	0	0	None
2 (First eluate)	80	48.2	48.2	Digitoxin only
3 (First eluate)	120	34.1	82.3	Digitoxin only
4 (First eluate)	160	9.7	92.0	Digitoxin only
5 (First eluate)	200	5.1	97.1	Digitoxin only
6 (First eluate)	240	2.7	99.8	Digitoxin only
7 (First eluate)	280	2.1	101.9	Digitoxin only
8 (First eluate)	320	1.0	102.9	Digitoxin only
1 (Second eluate)	20	0.9	103.8	Digitoxin only
2 (Second eluate)	40	0.6	104.4	Digitoxin only
3 (Second eluate)	60	1.1	105.5	Digitoxin (trace) Gitoxin
4 (Second eluate)	80	1.0	106.5	Gitoxin only
5 (Second eluate)	100	0	...	None

Similar results were obtained with two tablet preparations[1] of U. S. P. digitoxin reference standard, one containing bentonite in the formulation, the other containing starch. With the starch tablets 96% of the digitoxin was removed by 5 × 40 ml. of benzene-chloroform. The total recovery was 104%. The tablets containing bentonite gave 97% recovery of digitoxin with 200 ml. of the first eluate and a total recovery for all 13 fractions of 109%. These high recoveries are believed to be largely due to a small error, which is additive, in the colorimetric analysis. Evidence supporting this view was obtained by two further analyses of the bentonite tablets of U. S. P. digitoxin reference standard. The first five fractions were collected as before and the column was then eluted with 100 ml. of chloroform which was collected as one fraction. Analysis of these six fractions for digitoxin gave a total recovery of 102%. When all of the first eluate was collected as fraction 1 and the second eluate as fraction 2, analysis of the two fractions gave a total recovery of 100%. An error of 0.5% in the concentration of the solution of digitoxin used as a standard in the colorimetric analysis would account for the greater part of this high recovery. Any slight interference by nonglycoside components would also be magnified more than six times by this fractional assay.

On the basis of these recovery experiments the procedure was modified slightly. A total of 270 ml. of benzene-chloroform (3 + 1) was used as the first eluate. The first 25 ml. leaving the column was discarded, the remainder was collected in a 250-ml. volumetric flask, made up to the mark with chloroform and a 25-ml. aliquot was taken for colorimetric analysis. Chloroform, 100 ml., was used for the second eluate as before.

RESULTS AND DISCUSSION

The results of the digitoxin assays by the biological and chemical methods described above are compared in Table II. Figures shown in Column 1 represent the digitoxin found in the first eluate and are mean values of duplicate assays. The average difference between duplicates was less than 3%. The results are expressed as per cent potency in terms of U. S. P. digitoxin reference standard.

Of the 18 samples tested, 13 gave values by the U. S. P. XV method which were less than 90% of the corresponding figures by the biological assay (Column 4). Of interest was the fact that samples 4, 6, and 7 were different lots of tablets from one manufacturer and these three samples showed excellent agreement. In the case of sample 8 subsequent evidence indicated that the low value reported by the biological assay was due to an incomplete extraction of digitoxin from the tablets, presumably due to interference by tablet excipients. Based on the results using the U. S. P. XV method at least 11 of the 18 samples failed to conform to the U. S. P. assay requirements for digitoxin tablets. On the other hand only samples 3 and 8 were rejected by the official Canadian method of assay

[1] Obtained through the courtesy of Dr. L. C. Miller, Director of Revision, United States Pharmacopeia, New York, N. Y.

TABLE II.—COMPARISON OF THE U. S. P. XV CHEMICAL ASSAY AND THE OFFICIAL CANADIAN BIOLOGICAL ASSAY OF DIGITOXIN TABLETS

Sample No.	Per cent Potency in Terms of Standard —Chemical U. S. P. XV— Digitoxin Only	Modified for Total Glycosides	Biological (U. S. P. XIV Pigeon Method)	Chemical as Per Cent of Biological Column 1 vs. Column 3	Column 2 vs. Column 3
	(1)	(2)	(3)	(4)	(5)
1	81	96	96 ± 4.7	84	100
2	82	97	98 ± 3.3	85	99
3	65	86	75 ± 3.1	87	115
4	98	110	98 ± 4.1	100	112
5	90	98	100 ± 3.8	90	98
6	91	100	91 ± 5.6	100	110
7	94	101	93 ± 5.5	101	109
8	90	100	69 ± 3.0	130	145
9	90	101	105 ± 4.8	86	96
10	83	96	102 ± 5.0	81	94
11	87	93	104 ± 6.0	84	90
12	66	72	84 ± 5.0	79	86
13	85	95	106 ± 4.8	80	90
14	81	91	93 ± 3.8	87	98
15	79	89	94 ± 4.6	84	95
16	94	101	106 ± 5.4	89	95
17	80	87	100 ± 4.2	80	87
18	83	90	99 ± 4.7	84	91

which at the present time has a range of acceptance of ±20% of labeled strength.

The glycosides in the second eluate, identified by paper chromatography, have been shown to contribute to the biological assay, i. e., cardiac arrest in pigeons. The greater part of the glycosides present in the samples, in addition to digitoxin, consisted of gitoxin. Banes and Carol (8) calculated the toxicity for gitoxin as 40–45% of that for U. S. P. Digitoxin Reference Standard in pigeons. Gitoxin also contributes to the color reaction with alkaline picrate, giving a color about 80% as intense as that produced with digitoxin (9). Since the effect of gitoxin is included in the biological assay a better comparison between the two methods should result if gitoxin is also estimated in the chemical method even though the biological and the chemical responses are not equal in magnitude. Such a comparison is given in Table II, Columns 2 and 3. Here the values obtained in the biological assay are compared with those for total glycoside content, estimated as digitoxin, of the two eluates collected from the column in the chemical procedure.

On this basis only 2 of the 18 samples gave values by the chemical method which were less than 90% of those obtained by the biological assay (Column 5) Three samples gave results which were more than 110% of the corresponding biological assay figures. A second observation is that only four samples would fail to meet the requirements of U. S. P. XIV for digitoxin tablets (Column 2) compared to 11 that would be rejected on the basis of the U. S. P. XV procedure (Column 1).

The results of the qualitative analysis using paper chromatography indicated that the U. S. P. XV method was an efficient means of isolating the digitoxin in a pure form. In none of the 18 samples tested was there any trace of any glycoside, other than digotixin, in the first eluate even when as much as 320 ml. of the eluate was used. The separation also appeared to be sufficiently quantitative since the second eluate was free from digitoxin in 12 samples and in the remaining six it contained only traces of digitoxin, as estimated by the size and intensity of the spot appearing on the chromatogram.

SUMMARY

1. Comparative assays were carried out by the official Canadian method (U. S. P. XIV pigeon method) and by the U. S. P. XV method on 18 preparations of digitoxin tablets.

2. A slight modification of a preliminary draft of the U. S. P. XV method was found to give more accurate recoveries of digitoxin. This proved to be a satisfactory method for the estimation of the digitoxin content of a preparation.

3. The therapeutic and toxic effects of other cardiac glycosides, when present, are not accounted for by the U. S. P. XV method. An estimate of this residual glycoside content can be made by analysis of a chloroform eluate collected after the digitoxin has been isolated and probably should be done in order to indicate the amount of other glycosides which are present.

4. On the basis of this method 60 per cent of the digitoxin tablet preparations on the Canadian market would fail to meet assay requirements because of low digitoxin content.

5. To comply with the requirements of the U. S. P. XV monograph on digitoxin and digitoxin preparations, a higher purity of product will be required than is presently being attained by several manufacturers. This should be advantageous in securing better uniformity among digitoxin preparations.

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Modern Ointment Base Technology I.*

Properties of Hydrocarbon Gels

By MARGARET N. MUTIMER, CHARLES RIFFKIN, JOHN A. HILL, and GILMAN N. CYR

The disadvantages of petrolatum as an ointment base, i.e., greasiness, stickiness, and viscosity-temperature variations, can largely be overcome by the use of mineral oil gelled with certain waxes or resins, particularly polyethylene resin. The most desirable hydrocarbon gel appears to be one made by the very rapid chilling of a hot mixture consisting of 5 per cent polyethylene resin in U. S. P. liquid petrolatum. The critical factors in producing a good gel are: (*a*) the molecular weight of the polyethylene, (*b*) the percentage of polyethylene, (*c*) the viscosity of the mineral oil, and (*d*) the conditions of cooling.

THE OINTMENT base known as petrolatum is a by-product of the petroleum industry. It consists of highly purified extracts of lubricating oil residues. In structure, it is a two-phase colloidal system of oil and wax, the latter component comprising a crystalline portion and a "protosubstance" (1). This "protosubstance" is probably partially amorphous and acts as a gel former preventing the separation of the liquid from the solid hydrocarbon. It will be seen later how these elements contribute to the physical nature of the material.

Because petrolatum is of natural origin, it can be expected to vary somewhat with its source and the method by which it is refined. As an indication of this variability, the "United States Pharmacopeia" (2) recognizes petrolatums melting anywhere between 38–60°. Petrolatum of high melting point may be very sticky and difficult to spread whereas petrolatum of low melting point is liquid at some periods during the summer. Ointments made from the latter are apt to lose their original homogeneity.

However, petrolatum has enough advantages to have made it probably the most commonly used of all ointment bases. Extreme inertness, lack of skin sensitization, low cost, and ready availability are but a few of its advantages.

Petrolatum is a typical hydrocarbon gel. A study of it and other hydrocarbon gels will provide information indicative of the type of gel structure necessary for ideal ointment bases.

DISCUSSION

Wax-Thickened Mineral Oil.—It has already been stated that petrolatum consists of an oil component and a wax component. The latter fraction is responsible for the gel-like structure of petrolatum. It is however the least uniform part of the system; therefore, an investigation of other means of gelling this oil seems reasonable.

There are many materials which will thicken mineral oil. Among these are paraffin wax, amorphous wax, ozokerite, animal and vegetable waxes, and hydrocarbon polymers. The influence of each of these on mineral oil will be considered.

It is possible to alter the room temperature viscosity, and to some extent, the melting point of petrolatum by the addition of paraffin wax or oil, or both. This does not, however, improve the temperature-viscosity index. Moreover, when paraffin wax, which is completely crystalline, is dissolved in hot mineral oil alone and allowed to congeal, a granular mixture is the result. There is practically no gel structure formed, and as a consequence the product is neither smooth nor homogeneous. It is known that there is an amorphous wax fraction in petrolatum and that this exerts a profound effect on the gel structure formed by the paraffin wax portion. Attempts to gel mineral oil with an essentially amorphous wax results in a thickened or plasticized oil which is in no way satisfactory for use as an ointment base. The high degree of compatibility between the wax and oil yields a thick, heavy, stringy mass, and large amounts of wax must be

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used to reach the proper viscosity range of an ointment base. This is almost the exact opposite of the paraffin wax–mineral oil mixture. In one case there is no compatibility; in the other case, too much.

An interesting point in the behavior of wax gels is their thixotropy. If a gel shows a reversible decrease in viscosity when subjected to mechanical shear, it is said to be thixotropic. With reference to hydrocarbon gels, the presence of thixotropy indicates a gel structure of a stable, three-dimensional nature. Mineral oil can only be gelled by the addition of *both* crystalline material which adds rigidity to the mixture and a microcrystalline or amorphous material which produces a thickening effect. If the definitely crystalline portion is lacking, the mixture is not thixotropic.

Table I shows that the small amount of definite crystallinity contributed by paraffin produces a high initial viscosity, and that this crystallinity is responsible for forming the stable type of gel structure which exhibits thixotropy. The viscosity behavior of this mixture resembles that of petrolatum.

TABLE I.—EFFECT OF MILLING ON VISCOSITY OF WAX–MINERAL OIL MIXTURES

Product	Viscosity (Poises) Initial	After Milling at High Pressure Once	Twice
15% Multiwax W-445[a] in heavy mineral oil	1,400	1,400	1,400
10% Multiwax W-445 and 5% paraffin in heavy mineral oil	4,000	800	..
White petrolatum, U. S. P.	19,200	12,800	11,200

[a] A solid hydrocarbon mixture from heavy petroleum distillates and residues, Petroleum Specialties Company.

Ozokerite, an amorphous wax which is contaminated by crystalline portions, gels mineral oil quite well even in concentrations of the order of 5%. Being a natural product, it is subject to variation, including a wide melting point range and a variable degree of crystalline contamination. Both of these affect its use in ointment bases. The crystalline contamination in ozokerite, unsaturated and oxygenated compounds (3), make the wax less inert than other hydrocarbons and this is a disadvantage in itself. The behavior of ozokerite is further evidence that good stable gels are formed from thickeners containing part crystalline and part amorphous portions. Other waxes of this nature include animal and vegetable waxes like beeswax. These waxes are very sensitive to temperature changes and both harden and shrink when chilled. In addition, they are not hydrocarbons, and when used in ointment bases, hydrocarbon inertness is sacrificed.

Hydrocarbon polymers such as polyethylene produce a very satisfactory mineral oil gel when prepared properly. Since this type of compound is new in ointment base technology, a further description of the resultant product is indicated.

Hydrocarbon Polymers as Gelling Agents for Mineral Oil.—The above information indicates that of the available gelling agents a macromolecular hydrocarbon containing both crystalline and amorphous components would be most suited for producing the desired type of mineral oil gel. Polyethylene is a hydrocarbon of high molecular weight and high melting point. It contains both crystalline and amorphous fractions, which, as considered above, are prerequisites for the formation of optimal gel characteristics. Furthermore, polyethylene has a relatively flat temperature-viscosity curve and as such provides for good viscosity stability over a wide temperature range. It is a synthetic substance and hence subject to very close control during manufacture. Also it is available in a variety of molecular weights (4) as well as in different densities and varying degrees of crystallinity. Each of these properties actually plays a part in determining the final nature of the mineral oil gel and extreme importance must be given to these characteristics. For example, the importance of crystallinity can be seen in a solution of polyisobutylene in mineral oil. Polyisobutylene is a high molecular weight hydrocarbon polymer similar to polyethylene. It differs from the latter, however, in possessing no chain branching along the linear polymer. Since it is believed that chain branching is incident with crystallinity, it follows that polyisobutylene possesses no crystallinity. From this it would be predicted that a polyisobutylene–mineral oil gel would not give a gel structure such as that desired for the preparation of an ointment base. This is actually the case. A solution of polyisobutylene in mineral oil is a clear, viscous, sticky mass totally lacking in ointment-like properties.

Production of Mineral Oil–Polyethylene Gels (5).—In describing the procedure for making a polyethylene–mineral oil gel, the raw materials used are 21,000 mol. wt. polyethylene (Bakelite DYNH) and heavy mineral oil of 340-second viscosity. In every case, the finished gel will be of 5% resin concentration.[1]

Polyethylene dissolves in mineral oil when the mixture is heated at 130° for about three hours with good agitation. The resin itself melts at 110° and has a melt viscosity of a million poises. The hot solution is allowed to cool to a temperature just above its cloud point (95–100°) and it is then shock-cooled to a temperature just below its gel point (about 50°). Shock-cooling is a term used to describe a very rapid cooling of the hot melt. Cooling of the order of 10° per second is accomplished by spreading the hot solution in a thin film on a metal surface which is water cooled to a constant temperature, and removing the film from the surface when it reaches 50°. Rapid chilling is necessary to produce the gel structure which is probably accomplished by supercooling the polyethylene to below its cloud point, all the resin then precipitating colloidally very rapidly. The insolubility of polyethylene in mineral oil below this temperature insures no subsequent re-solution of the resin in the oil, and in this manner, excellent temperature stability of the gel is achieved. The viscosity of the gel appears to be little changed at 5° or at 45°. This characteristic, of course, is quite different from that of the preparation resulting when polyethylene and mineral oil are melted together and slowly cooled. Under these conditions, a grainy, slushy mixture is obtained which is soft enough to pour and contains hard lumps of resin.

[1] This product is available commercially as Plastibase. Plastibase is a Squibb trademark.

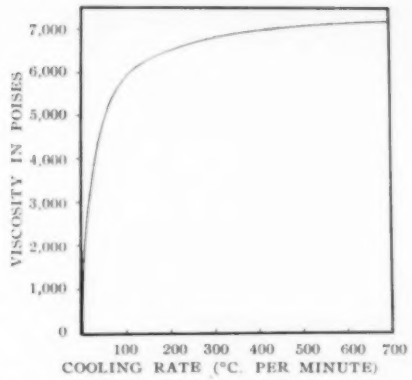

Fig. 1.—Effect of cooling rate on viscosity of gel consisting of heavy mineral oil with 5 per cent polyethylene (DYNH).

By increasing the rate of cooling to an optimum point, the viscosity of the mixture rises (Fig. 1), thixotropy is increased, and the gel becomes uniform, soft, and glossy.

Theory of the Gel Structure.—A three-dimensional lattice structure has been proposed to describe the physical nature of polyethylene gels. Since polyethylene precipitates as small crystallites surrounded by long filaments of amorphous, fibrous resin, as seen in the phase microscope, this seems to be a logical theory (6). At a 5% resin concentration in oil, these small crystallites are just close enough together to allow the long fibers to intermesh and produce a sponge-like structure which entraps and holds the oil. The number of small crystallites present in a 5% gel is determined partly by the shock-cooling process and partly by the degree of crystallinity of the polyethylene. The oil phase of the gel is thought to be mobile. Theoretically, the movement of the oil through the gel structure brings a continuously changing interface of medication to the skin, when used as an ointment base, and thereby permits a release of active ingredients so much superior to petrolatum as to approach the emulsion type bases. While the theory of oil mobility is difficult to demonstrate, the rate of drug release can be dramatically shown.

Selection of Most Suitable Resin.—Polyethylene resin is available in molecular weights ranging from 1,000 to 38,000 and in a melting point range of 37.5–112° C. Contrast this with the maximum molecular weight for paraffin wax which is 575 with a melting point of 180° F. The degree of crystallinity in polyethylene varies with the polymerization process and several varieties are available.

Bakelite DYNH polyethylene (4) has a molecular weight of 21,000, a melting point of 110°, 50–51% crystallinity, melt viscosity of 1,000,000 poises, and a density of 0.92.

The selection of the proper polyethylene resin or combination of resins in the right concentration to gel mineral oil was determined experimentally. It was stated above, that for a stable gel it is necessary to have a certain amount of crystalline gelling agent and the proper ratio of amorphous, fibrous, oil-holding wax. It was found that polyethylene resins with molecular weight lower than 12,000 may have the proper per cent crystallinity, but the ratio of amorphous resin surrounding each crystallite is not sufficient to produce a strong, stable gel. The oil-holding power of the low molecular weight resin is likewise less than that of the higher molecular weight resin. Polyethylenes are also available with a molecular weight higher than 25,000 but these are also not satisfactory because mineral oil is such a poor solvent for polyethylene that more than three hours heating is needed to dissolve these very high molecular weight resins. This excessive heating tends to crack the mineral oil. Also, once the high molecular weight resins are dissolved and shock-cooled, the gels they produce are no better than those from the 21,000 molecular weight resin.

The selection of a polyethylene with the optimum degree of crystallinity for a good gel is governed by limitations in the range of crystallinity available in commercial polyethylene and also by the method of shock-cooling the gel. While polyethylene of any given degree of crystallinity is in solution in hot mineral oil it is devoid of all crystallinity. The rate of shock-cooling of this solution governs to some extent the degree of crystallinity which is restored and therefore the stability and appearance of the gel. Slower shock-cooling restores more crystallinity which makes for granular gels whereas extremely rapid shock-cooling produces extremely tacky and unstable gels. It is apparent that closely controlled conditions are necessary to produce a consistently desirable gel structure.

The mineral oil phase of the gel can also be varied, but with little change in the appearance and stability of the gel since mineral oil exhibits a poor temperature-viscosity behavior. A blend of oils of different viscosities or a mixture of oil and 1–2% polyisobutylene tends to stabilize oil viscosity which makes for better gel stability at elevated temperatures.

Polymer Gel Stability.—The chemical stability of mineral oil–polyethylene gels is obvious from the nature of the raw materials. Polyethylene is extremely inert, reacting with nothing in the cold. When heated above its melting point, its only possible reaction at that temperature is a very slight tendency for oxidation, and this tendency is so small that antioxidants are unnecessary in processing mineral oil–polyethylene gels. Mineral oil is almost as inert as polyethylene. Its chemical behavior is familiar to everyone. Thus, the chemical stability of ointments prepared from mineral oil–polyethylene gels is entirely dependent on the active ingredient, as there is little possibility for reaction between the base and the active ingredient.

Since mineral oil gelled with polyethylene has a much lower "wax" concentration than petrolatum, a much larger percentage of solids can be added to it than to petrolatum. Twenty per cent zinc oxide, for example, when added to a mineral oil-polyethylene gel produces a soft, smooth ointment. This ointment has practically the same consistency at room temperature as at 5 or 40°. Twenty per cent zinc oxide in petrolatum, however, is a stiff, heavy paste. Since the viscosity of polyethylene gels does not significantly change by altering the temperature (Fig. 2), there is no problem with settling of the active ingredient. This is a serious disadvantage with petrolatum-based ointments (7).

Mineral oil is compatible with a wide variety of compounds. And so is mineral oil gelled with poly-

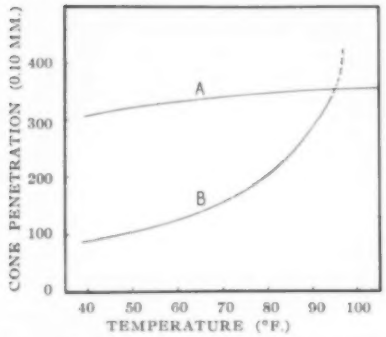

Fig. 2.—Effect of temperature on cone penetration reading on Plastibase (A) and White Petrolatum (B).

ethylene. Compatibility for ointment purposes ranges from unreactive materials like starch and zinc oxide to pine tar and methyl salicylate. Antibiotics such as penicillin have better stability in polyethylene-gelled mineral oil than in a base of white petrolatum, peanut oil, anhydrous lanolin, and white beeswax (Fig. 3 and 4).

The physical stability of polyethylene-gelled mineral oil is reflected in the temperature-viscosity stability. Since the gel is slightly thixotropic, excessive shear causes a reduction in viscosity which is only partly reversible. The sensitivity to shear, while not serious, can be alleviated somewhat by the incorporation of some highly thixotropic wax or some inorganic filler. However, some of the extreme inertness of the gel would be sacrificed if this were done, and the gel is not sufficiently sensitive to shear to warrant any change that may affect the chemical stability. Additional polyethylene resin (above 5%) makes an inert gel that is more resistant to shear, but with increased viscosity and a proportionally lower rate of release of active ingredient.

Polyethylene gels are sufficiently resistant to mechanical working and shear to allow for cold compounding. The ointments can be mixed, milled, and mechanically packaged with very little, if any, effect on the gel stability. There is no reason to use heat for any formulation involving these gels since their consistency is such that compounding may be easily carried out at room temperature, or lower.

Additives can be incorporated into polyethylene-mineral oil gels to modify some of their characteristics. For example, oil-soluble emulsifiers can be added to the solution just before shock-cooling. Glyceryl mono-oleate, at a 6% level, will impart absorption properties. Water can then be added at room temperature to form a stable water in oil emulsion. The properties of polyethylene influence the stability of such an emulsion. It is viscosity-stable over a wide temperature range.

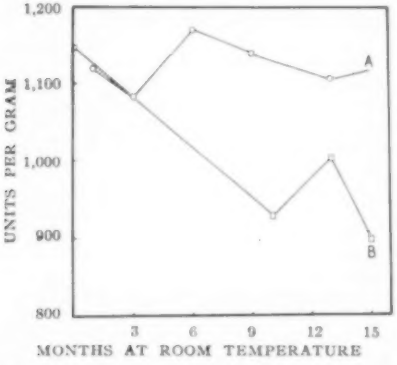

Fig. 3.—Stability of penicillin G potassium in Plastibase (A) and in a white petrolatum, peanut oil, anhydrous lanolin, white beeswax base (B).

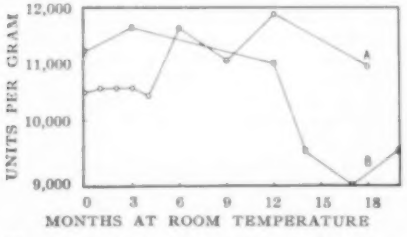

Fig. 4.—Stability of Penicillin G Potassium in Plastibase (A) and in a white petrolatum, peanut oil, anhydrous lanolin, white beeswax base (B).

SUMMARY

Petrolatum is probably the most widely used ointment base. Because it is a natural product and subject to variations in methods of refinement, the official product may vary from a hard, sticky grease to a very soft low-melting gel. This is due to variations in the relative proportion of oil/crystalline wax/amorphous wax. Hydrocarbon gels can be formed by the addition of various waxes to mineral oil. It is shown that good hydrocarbon gels result only with optimum proportions of crystalline and amorphous wax dispersed in the oil phase. For chemical inertness, the waxes and oil should preferably be hydrocarbon in nature.

Polyethylene, a hydrocarbon, is capable of forming a gel with mineral oil when properly treated. The best gel is formed when polyethylene having a molecular weight of 21,000 is dissolved in heavy mineral oil of 340-second viscosity and the solution cooled at the rate of about 10° per second from just above its cloud point to just below its gel point. The polyethylene precipitates as small crystallites surrounded by long filaments of amorphous resin. These small crystallites are close enough to allow the long fibers to intermesh and produce a sponge-like structure which holds the oil.

This polyethylene–mineral oil gel is chemically extremely stable. It maintains a very desirable, soft, ointment consistency over a wide temperature range (−15° to 60°). Its range of compatibility is very broad. Additives can be incorporated to impart absorption properties.

Polyethylene–mineral oil gels are presented as a significant improvement over the conventional hydrocarbon ointment bases.

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A Study of Some Factors Influencing the Stability of Pharmaceutical Emulsions I.*

The Effect of Two-Stage High Pressure Homogenization

By JOHN D. MULLINS† and CHARLES H. BECKER

The stability of oil-in-water emulsions prepared from four oils has been investigated. Emulsions were stabilized with nonionic emulsifiers and with acacia. The effect of homogenization pressure was studied by passing all emulsions through a Manton-Gaulin homogenizer. Stability characteristics were evaluated by means of a size frequency method of analysis. Results indicate that specific interfacial area increases at a constant rate with increased homogenization pressure. Deterioration characteristics of the emulsions studied were found to be dependent primarily on the type of emulsifier used and, to a lesser extent, on the oil constituting the internal phase. Stability and homogeneity increased with homogenization pressure. Viscosity was not influenced.

EMULSION STABILITY and factors influencing stability have been the subject of many investigations. However, because of the various methods of preparing and stabilizing emulsions, as well as the many methods of evaluating stability, much of the literature is difficult to interpret and correlate. In this investigation various homogenization pressures were used to see if an increase in pressure enhanced emulsion stability. The effect of increased homogenization pressure on viscosity of the emulsion and specific interfacial area were also noted.

EXPERIMENTAL

Oils.—Four oils were selected for use in this investigation. Both the cod liver and peanut oils were of U. S. P. grade. Glyceryl trioleate (Lot No. 2230) was purchased from Emery Industries, while glyceryl ricinoleate (Lot No. 30989, Neutral) was obtained from Kessler Chemical Company. Some of the physical constants of these oils were determined and are listed in Table I.

TABLE I.—PHYSICAL CONSTANTS OF OILS AT 30° C.

Oil	Specific Gravity	Viscosity, c. p. s.	Interfacial Tension dynes/cm.[1]
Peanut	0.9120	59	23.6
Cod liver	0.9219	44	9.5
Glyceryl ricinoleate	0.9853	522	5.9
Glyceryl trioleate	0.9123	54	11.9

Emulsifying Agents.—Acacia and a combination of nonionic emulsifying agents were selected for use in this work. Acacia was used at a concentration of 2.5%. The nonionic agents, Tween® 80 and Arlacel® C (Atlas Powder Company), polyoxyethylene sorbitan mono-oleate and sorbitan sesquioleate, respectively, were used together in ratios totaling 1.0%. The HLB system (1), hydrophylic-lipophilic balance, of classifying and selecting emulsifiers was employed to determine the proportion of each nonionic agent to be used. The optimum HLB values for the various oils were determined experimentally, and these values, together with the relative proportions of Tween® 80 and Arlacel® C required are listed in Table II.

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Abstracted from a dissertation presented to the Graduate Council of the University of Florida by John D. Mullins in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
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TABLE II.—OPTIMUM HLB VALUES AND RELATIVE PROPORTIONS OF TWEEN® 80 AND ARLACEL® C REQUIRED

Oils	Optimum HLB	Tween® 80 (Required Proportions)	Arlacel® C (Required Proportions)
Peanut	8.0	38	62
Cod liver	7.5	34	66
Glyceryl trioleate	9.5	52	48
Glyceryl ricinoleate	11.5	69	31

External Phase.—Distilled water containing 0.1% methylparaben and 0.025% propylparaben was used.

Homogenization.—After premixing, all emulsions were passed twice through a Manton-Gaulin two-stage homogenizer Model B. The use of a two-stage homogenization process is said to result in less coalescence of the internal phase and a final product of lower viscosity (2). Four emulsions were prepared from each oil to study the effect of increased homogenization pressure on stability.

Four different two-stage pressure settings were used. The first homogenizing valve was set at pressures of 1,500, 2,000, 2,500, and 3,000 pounds per square inch. The second valve was set at 1,000 pounds per square inch in all cases. To assure uniformity, all pressure settings were obtained by adjusting the machine on water. When the homogenizer was operating correctly at the desired pressure settings, the water was diverted to a container. When the water had nearly disappeared from the intake pipe the premixed emulsion was added. Although the Manton-Gaulin homogenizer is equipped for recycling, this procedure was avoided and two separate passes were used. The machine was well rinsed between passes and pressure built up as described above.

Preparation of Emulsions.—Emulsions of all oils were prepared at a concentration of 25% v/v without the use of heat. A total volume of 400 cc. was selected as a convenient amount for homogenization. In the case of the Tween® 80-Arlacel® C emulsions, these nonionic emulsifiers were first dissolved in the oil. The acacia was first dissolved in the water in the case of the acacia emulsions. The external phase was added to the oil phase in divided portions. Each addition was followed by agitation sufficient to achieve partial emulsification. When the two phases were combined the system was passed twice through the Manton-Gaulin homogenizer as described above.

Procedure of Size Frequency Method of Analysis.—A standard hemocytometer cell having a depth of 0.1 mm. was used in conjunction with a thin glass cover slip to facilitate focusing. The hemocytometer cell rulings were not involved in the analysis, however, a ruled glass disk was inserted in the microscope ocular to aid in counting.

Propylene glycol was selected as a diluting fluid component due to its chemical stability and relatively high specific gravity. In addition, it has no inherent emulsifying action (3) and thus prevented any possibility of further emulsification of the sample upon dilution. A 75% aqueous solution retarded Brownian movement to a satisfactory degree and caused a high percentage of the dispersed globules to rise to a single plane. In general, the dilution varied from approximately 1:150 to 1:200 depending on the emulsifier used and the pressure of homogenization.

Microscope apparatus included an American Optical Co. camera lucida microscope attachment in conjunction with a ×97 oil immersion objective and a ×15 ocular. The total magnification was thus ×1455. Calibration of the optical system with a slide micrometer indicated that 2 mm. on the drawing paper was equivalent to one micron at the above magnification.

Previous emulsion investigations involving a size frequency method of analysis have been based on the measured diameters of approximately 1,000 oil globules although a minimum sample of 400 has been said to yield reproducible results (3). The magnitude of sample size in this investigation was based on the number necessary to give a definite probability of success based on an emulsion of lowest homogeneity. It was found that approximately 900 globules must be measured to achieve reproducible results within the level of probability used. In practice 900 was considered an approximate minimum count.

A portion of the diluted sample was placed in the hemocytometer cell and the outlines of approximately 1,000 oil globules were traced. The entire cell depth of each field was carefully searched by refocusing the microscope. When the drawing was complete, each globule was measured and its diameter was recorded. The actual diameters were not required since class limits of one-micron units were selected and the globule diameters were recorded as frequency of occurrence within class limits. For purposes of calculation the class midpoints were selected as representing the average of each class.

The volumes and areas of globules having the midpoint diameters were calculated (as spheres) and expressed as cc. and square cm., respectively. These constants, when multiplied by the class frequency, yielded the volume or area of the number of oil globules of a specific diameter. In this manner the sum of the calculated volumes and the sum of the calculated areas permitted the determination of area per unit volume.

Viscosity Measurements.—All determinations were taken immediately after homogenization and at a temperature of 30°. An Ostwald viscosimeter having a capacity of 2 cc. was used.

Storage of Emulsions.—All emulsions were subdivided and stored in 4-oz. clear glass bottles. Samples were stored at room temperature and in an electric oven at 45°. Size frequency analyses were performed on all samples immediately after homogenization and at intervals of 15, 30, and 60 days.

Effect of Pressure on Specific Interfacial Area.—The results illustrated in Fig. 1 and 2 show that specific interfacial area increased at a constant rate with increased homogenization pressure. The rate of increase was similar for all emulsions containing a common emulsifier. The initial magnitude of specific interfacial area could not be correlated with the viscosity or interfacial tension of the individual oils.

Emulsions Stabilized with Tween® 80 and Arlacel® C.—The data listed in Table III and illus-

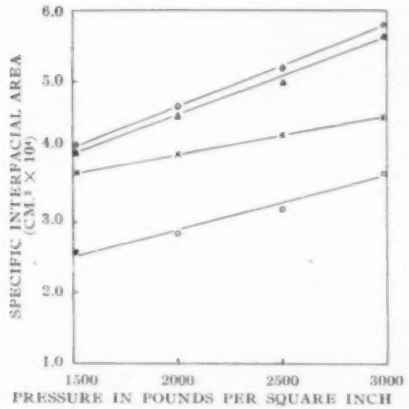

Fig. 1.—The effect of homogenization pressure on specific interfacial area in emulsions stabilized with Tween® 80 and Arlacel® C. O—Glyceryl ricinoleate, ×—peanut oil, ◇—glyceryl trioleate, △—cod liver oil.

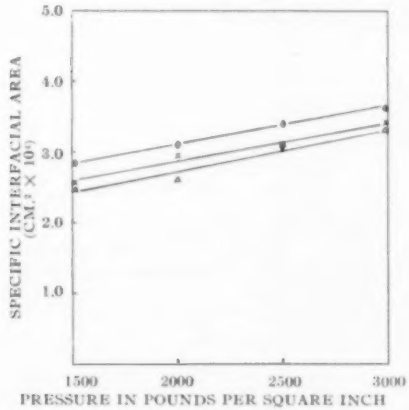

Fig. 2.—The effect of pressure on specific interfacial area in emulsions stabilized with acacia. O—Peanut oil, ×—glyceryl trioleate, ◇—cod liver oil.

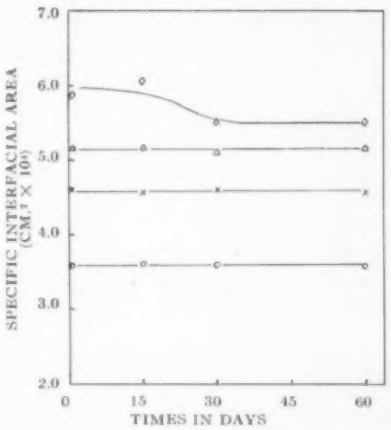

Fig. 3.—Variation in specific interfacial area of glyceryl trioleate emulsions stabilized with Tween® 80 and Arlacel® C and stored at room temperature. Homogenization pressures, lb. per sq. inch: O—1500, ×—2000, △—2500, ◇—3000.

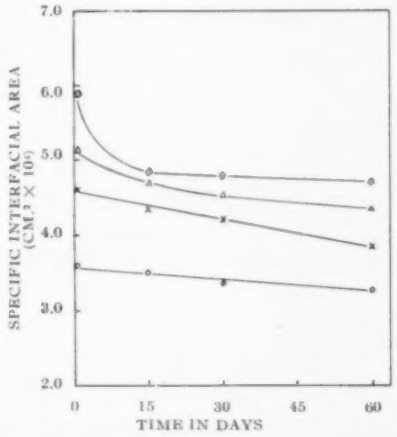

Fig. 4.—Variation in specific interfacial area of glyceryl trioleate emulsions stabilized with Tween® 80 and arlacel® C and stored at 45° C. Homogenization Pressures, lb. per sq. inch: O—1500, ×—2000, △—2500, ◇—3000.

trated in Fig. 3 and 4 show the effect of storage temperatures on specific interfacial area in glyceryl trioleate emulsions stabilized with Tween® 80 and Arlacel® C. In general, deterioration in the emulsions stabilized with the nonionic emulsifiers was approximately linear with respect to time. The abrupt decrease in interfacial area in the emulsion homogenized at 3,000 lb. may have been due to a lack of sufficient emulsifier resulting in a decrease in specific interfacial area to form a more stable condensed monomolecular film. Subsequent to this change in interfacial area the deterioration was linear with respect to time.

Results obtained with other oils in this investigation were somewhat similar to the results obtained with glyceryl trioleate. It was apparent that deterioration by globule coalescence took place only to a limited extent since free oil was present in many cases even though globules greater than 7–8 μ in diameter were very rare. This is in accord with results obtained by King and Mukherjee (4) in their work on soap stabilized emulsions. The variation in results obtained with other oils also indicated that the type of deterioration was influenced to some extent by the character of the internal phase in addition to the type of emulsifier used.

Emulsions Stabilized with Acacia.—The results listed in Table IV and illustrated in Fig. 5 and 6 show the effect of storage on the specific interfacial area in emulsions stabilized with acacia. Interfacial area decreased at a decreasing rate with time. Apparently deterioration in these emulsions took place

TABLE III.—GLYCERYL TRIOLEATE EMULSIONS STABILIZED WITH TWEEN® 80 AND ARLACEL® C—FREQUENCY DISTRIBUTIONS

Room Temperature Storage[a]

Diameter (Microns)	Homogenization Pressures (lb./sq. in.) 1500 Time in Days 0	15	30	2000 Time in Days 0	15	30	2500 Time in Days 0	15	30	3000 Time in Days 0	15	30
0.5	1035	1295	1123	1401	841	1176	878	1085	1389	1136	1046	1289
1.5	117	132	122	116	73	105	113	78	146	59	48	104
2.5	17	26	17	10	12	22	7	7	15	6	4	11
3.5	4	7	7	1	2	3	..	1	..	1	1	..
4.5	3	..	2	2	..	..	..	..	..	..	..	..
5.5		1										
Totals	1176	1461	1271	1530	928	1306	998	1171	1550	1202	1099	1404
S[b]	3.58	3.64	3.62	4.62	4.56	4.62	5.18	5.15	5.11	5.86	6.11	5.50

Storage at 45°[a]

Diameter (Microns)	Homogenization Pressures (lb./sq. in.) 1500 Time in Days 0	15	30	2000 Time in Days 0	15	30	2500 Time in Days 0	15	30	3000 Time in Days 0	15	30
0.5		577	899		989	1012		1107	1069		1008	1049
1.5		84	163		111	115		79	118		77	92
2.5		14	22		11	20		7	11		13	5
3.5		4	9		2	3		2	1		2	4
4.5		1	2		1	..		1	1		..	..
Totals		680	1095		1114	1150		1196	1200		1100	1150
S[b]		3.50	3.35		4.32	4.22		4.70	4.53		4.72	4.76

[a] Sixty-day storage values were omitted. [b] Specific interfacial area (cm.^{2} × 10^4).

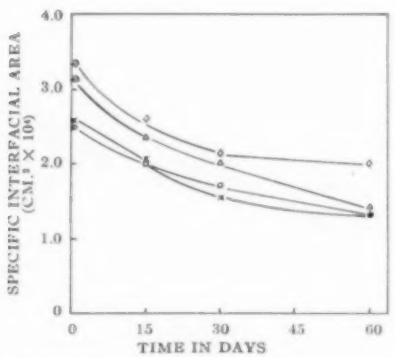

Fig. 5.—Variation in specific interfacial area of glyceryl trioleate emulsions stabilized with acacia and stored at room temperature. Homogenization pressures, lb. per sq. inch: O—1500, ×—2000, △—2500, ◇—3000.

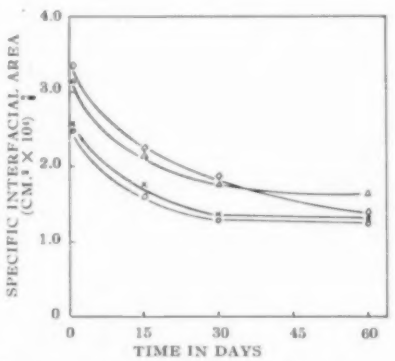

Fig. 6.—Variation in specific interfacial area of glyceryl trioleate emulsions stabilized with acacia and stored at 45° C. Homogenization pressures, lb. per sq. inch: O—1500, ×—2000, △—2500, ◇—3000.

only by coalescence of the internal phase globules since free oil was observed in only cod liver oil emulsions after 60 days' storage. In general, the magnitude of specific interfacial area in acacia stabilized emulsions was smaller than in the corresponding emulsions stabilized with Tween® 80 and Arlacel® C. This was due to the increased frequency of relatively large oil globules in the acacia-stabilized emulsions.

DISCUSSION

The size frequency method of analysis as used in this investigation, while time consuming and subject to certain errors, is, in the opinion of many investigators, the most reliable means of evaluating emulsion stability. Duplicate samples prepared during this investigation showed a maximum error of about 5% although the average error was approximately 2–3%. In addition, changes in the frequency distribution which did not influence the mean particle size caused definite changes in specific interfacial area.

Emulsions Stabilized with Tween® 80 and Arlacel® C.—Although deterioration in these emulsions was approximately linear with respect to time it would be difficult to predict stability on this basis because of the separation of free oil. All of the emulsions in this section showed a maximum in the particle size distribution at 0.5 μ. Thus, it may be concluded that the viscosity of the internal phase has no influence on the particle size distribution.

TABLE IV.—GLYCERYL TRIOLEATE EMULSIONS STABILIZED WITH ACACIA—FREQUENCY DISTRIBUTIONS

Room Temperature Storage[a]
Homogenization Pressures (lb./sq. in.)

Diameter (Microns)	1500 Time in Days 0	15	30	2000 Time in Days 0	15	30	2500 Time in Days 0	15	30	3000 Time in Days 0	15	30
0.5	1107	1085	1276	1132	1351	959	1038	1346	1174	1151	1121	1358
1.5	243	184	153	264	257	141	174	164	135	170	116	137
2.5	70	46	33	77	67	41	28	33	25	36	24	23
3.5	24	10	5	19	17	6	8	8	7	12	4	4
4.5	7	3	1	3	6	1	5	2	2	1	2	3
5.5	2	3	1	4	2	2	..	2	2	..	2	1
6.5	1	2	2	1	2	2	..	1	1	..	2	3
7.5	..	..	1	..	4	3	..	2	2	..	..	2
8.5	..	..	..	..	..	..						
9.5	..	..	1	..	..	2						
10.5	..	..	..	..	..	..						
Totals	1454	1333	1473	1500	1706	1157	1253	1558	1348	1370	1271	1531
S[b]	2.49	1.98	2.08	2.56	2.05	1.55	3.15	2.37	2.02	3.36	2.60	2.14

Storage at 45°[a]
Homogenization Pressures (lb./sq. in.)

Diameter (Microns)	1500 Time in Days 0	15	30	2000 Time in Days 0	15	30	2500 Time in Days 0	15	30	3000 Time in Days 0	15	30
0.5	1107	996	1056	1132	953	906	1038	1167	1223	1151	944	1108
1.5	243	174	79	264	184	111	174	134	96	170	109	80
2.5	70	44	24	77	53	25	28	33	35	36	17	18
3.5	24	10	10	19	20	7	8	13	14	12	10	11
4.5	7	4	4	3	8	3	5	7	1	1	4	8
5.5	2	5	5	4	6	4	..	3	5	..	2	3
6.5	1	6	2	1	1	3	..	3	4	..	..	1
7.5	..	..	3	..	1	3	..	..	1	..	..	2
8.5	..	1	1	..	2	2	..	..	1	..	1	..
9.5	..	..	..	..	..	..						
10.5	..	1	2	..	..	1						
Totals	1454	1241	1186	1500	1228	1065	1253	1360	1380	1370	1087	1231
S[b]	2.49	1.59	1.25	2.56	1.51	1.32	3.15	2.17	1.77	3.36	2.22	1.88

[a] Sixty-day storage values omitted. [b] Specific interfacial area (cm.^{2} × 10^4).

This is in accord with results obtained by Berkman (5). In general, it was found that the rate of decrease in specific interfacial area was greater in emulsions stored at 45° than in samples stored at room temperature.

Emulsions Stabilized with Acacia.—Results obtained in this section indicated that while deterioration was somewhat similar in all of the oils tested the properties of the individual oils could influence the type of emulsion deterioration. In general, however, deterioration was characterized by a sharp decrease in specific interfacial area followed by a period of relatively good stability. As previously found, the rate of deterioration was greater in emulsions stored at 45°. This is in agreement with results obtained by Levius and Drummond (3). It should be mentioned that creaming occurred in all of the emulsions tested regardless of the emulsifying agent. The creamed portions were usually slight and were easily redistributed by gentle shaking.

SUMMARY

1. The stability characteristics of oil-in-water emulsions prepared from four oils have been studied. Emulsions of each oil were prepared with two emulsifying agents, acacia and blends of Tween® 80 and Arlacel® C.

2. The effect of two-stage homogenization on stability characteristics has been investigated. A size frequency method of analysis was used to evaluate stability.

3. The magnitude of specific interfacial area increased at a constant rate with increased homogenization pressure. The increase was similar regardless of the oil constituting the internal phase.

4. In emulsions stabilized with Tween 80® and Arlacel® C, decrease in specific interfacial area was found to be approximately linear with respect to time.

5. In emulsions stabilized with Tween® 80 and Arlacel® C deterioration by globule coalescence took place only to a limited extent. Free oil was observed in many cases even though globules of the internal phase greater than 7–8 microns in diameter were very rare.

6. Emulsions stabilized with acacia were characterized by comparatively lower specific interfacial area than in the corresponding Tween-Arlacel stabilized emulsions. This was due to the increased frequency of relatively large oil globules.

7. Deterioration in acacia-stabilized emulsions took place at a decreasing rate with time. With

one exception, deterioration was accomplished by globule coalescence only. Free oil was observed in cod liver oil emulsions after 60 days' storage.

8. The viscosity of the emulsions studied was not influenced by changes in specific interfacial area nor constituents of the internal phase. Viscosity did vary with the emulsifier used.

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A Study of Some Factors Influencing the Stability of Pharmaceutical Emulsions II.*

The Effect of Wax and Specific Gravity Adjustment

By JOHN D. MULLINS† and CHARLES H. BECKER

Oil-beeswax emulsions and emulsions in which the specific gravity of the internal phase was adjusted to equal that of the external phase were investigated. A size frequency method of analysis was used to evaluate stability. Results indicated that beeswax had no effect on emulsion stability. Adjustment of specific gravity decreased specific interfacial area but increased stability in some instances.

IN A PREVIOUS PAPER (1) it was shown that the stability and homogeneity of the emulsions studied increased with homogenization pressure. In the present investigation, some emulsions were prepared in which the specific gravity of the internal phase was adjusted to equal that of the external phase to see if this factor would promote emulsion stability. In addition, some emulsions were prepared in which the oil phase was rendered thixotropic with wax to ascertain if this would enhance emulsion stability.

EXPERIMENTAL

Emulsion constituents, concentration, storage time and temperature, method of preparation, and the size frequency method of evaluating stability are the same as described in a previous investigation (1) except as indicated below.

Emulsions of Oil-Beeswax Systems Stabilized with Tween® 80 and Arlacel® C.—Peanut oil and glyceryl ricinoleate were used in this portion of the work. An amount of beeswax, 4%, sufficient to impart thixotropy was added to these oils. Since the thixotropic system peanut oil-beeswax could not be emulsified at room temperature because of wax separation, both premixing and homogenization had to be carried out at a temperature of approximately 75°. At this temperature a stable emulsion of high homogeneity was obtained. However, microscopic examination of these emulsions showed that many of the internal phase globules were present as aggregates. This aggregation and resultant distortion of the globules effectively prevented size frequency analysis.

Mixtures of glyceryl ricinoleate and beeswax could not be satisfactorily emulsified regardless of wax concentration, emulsifier concentration, or temperature.

Emulsions of Oil-Beeswax Systems Stabilized with Acacia.—Glyceryl trioleate and cod liver oil were selected for use in this section. As described previously, these emulsions had to be prepared by premixing and homogenizing at a temperature of approximately 75°. In the homogenization process correct pressures were obtained by adjusting the Manton-Gaulin homogenizer with hot water.

Emulsions of Uniform Specific Gravity Stabilized with Tween® 80 and Arlacel® C.—To investigate the effect of uniform specific gravity on emulsion stability the internal phases of emulsions prepared from peanut oil and glyceryl ricinoleate were adjusted to equal the specific gravity of the external phase. Since the maximum concentration of nonionic emulsifier used here did not appreciably alter the specific gravity of the preserved water it was considered sufficiently accurate to adjust the specific gravity of the oils to 1.000. The desired specific gravity for both oils was obtained by the addition of brominated oil having a specific gravity of 1.330. This brominated oil was of food grade and purchased from H. Kohnstamm and Company, Inc.

The optimum HLB value for brominated oil, obtained experimentally, was 8.0. Thus, no alteration in the Tween-Arlacel ratio was required for peanut oil–brominated oil mixtures. The optimum HLB for glyceryl ricinoleate–brominated oil mixture was altered slightly to 11.4. This value was ob-

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TABLE I.—GLYCERYL TRIOLEATE-BEESWAX EMULSIONS STABILIZED WITH ACACIA AND STORED AT ROOM TEMPERATURE

Diameter (Microns)	Frequency Distributions Pressure—Pounds per Square Inch 1500 Time in Days 0	15	30	60	2000 Time in Days 0	15	30	60
0.5	1441	1076	1241	1285	1334	858	1010	1190
1.5	137	96	166	172	112	117	115	166
2.5	19	22	31	31	20	13	19	29
3.5	4	5	6	7	2	3	3	5
4.5	..	..	..	1	..	..	..	2
Totals	1601	1199	1444	1496	1468	991	1147	1392
S[a]	4.52	4.05	3.91	3.72	4.69	4.30	4.24	3.68

[a] Specific interfacial area in $cm.^2 \times 10^4$.

TABLE II.—GLYCERYL TRIOLEATE EMULSIONS STABILIZED WITH ACACIA AND STORED AT ROOM TEMPERATURE

Diameter (Microns)	Frequency Distributions Pressure—Pounds per Square Inch 1500 Time in Days 0	15	30	60	2000 Time in Days 0	15	30	60
0.5	1241	1069	960	1130	1319	949	1291	873
1.5	122	95	92	104	107	121	173	120
2.5	16	22	24	33	16	22	30	20
3.5	3	5	4	5	3	..	3	4
4.5	..	..	..	1	..	..	..	1
Totals	1382	1191	1080	1273	1445	1092	1497	1018
S[a]	4.57	4.08	4.03	3.67	4.71	4.45	4.19	3.76

[a] Specific interfacial area in $cm.^2 \times 10^4$.

tained by calculation making use of the additive property of HLB numbers.

Emulsions of Uniform Specific Gravity Stabilized with Acacia.—Glyceryl trioleate and cod liver oil were used in this study. As before, specific gravity was adjusted to equal that of the external phase, in this case a solution of acacia having a specific gravity of 1.011. Brominated oil was used to obtain the required specific gravity for both oils.

DISCUSSION

Emulsions of Oil-Wax Systems Stabilized with Tween® 80 and Arlacel® C.—As previously noted, emulsions of this type could be prepared only by the use of high temperatures. In addition, globule aggregation effectively prevented size frequency analysis. This condition was apparently due to the type of emulsifier used since similar results were obtained when glyceryl trioleate or cod liver oil–wax systems were emulsified with Tween® 80 and Arlacel® C.

Emulsions of Oil-Wax Systems Stabilized with Acacia.—Preliminary results indicated that the addition of beeswax to the internal phase offered no apparent advantages. To evaluate these emulsions more accurately a series of glyceryl trioleate emulsions containing no added beeswax was prepared by premixing and homogenizing at a temperature of 75°. From the results obtained it may be concluded that increases in specific interfacial area were caused by the higher temperature used and not by the wax present in the internal phase.

To determine any effect of beeswax on stability the series described above was stored at room temperature for a 60-day period with size frequency analyses taken at the stated intervals. The results listed in Tables I and II and illustrated in Fig. 1 and 2 indicated that the rate of change in specific inter-

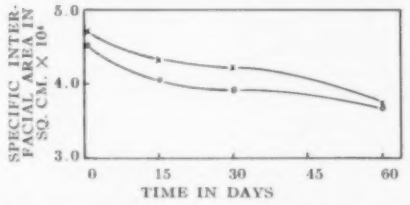

Fig. 1.—Variation in specific interfacial area of glyceryl trioleate-beeswax emulsions stabilized with acacia and stored at room temperature. Homogenization pressures, p. s. i.; O—1500, ×—2000.

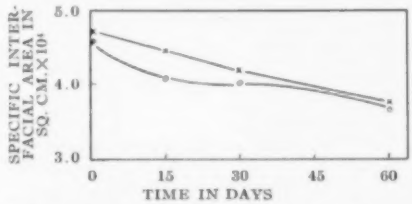

Fig. 2.—Variation in specific interfacial area of glyceryl trioleate emulsions stabilized with acacia, homogenized at 75° C. and stored at room temperature. Homogenization pressures, p. s. i.; O—1500, ×—2000.

facial area was less than that obtained with the control emulsions of glyceryl trioleate stabilized with acacia. However, the decrease in rate was due mainly to the reduction in particle size caused by heat since the rate curves obtained for emulsions with and without beeswax are approximately parallel.

Emulsions of Uniform Specific Gravity Stabilized with Tween® 80 and Arlacel® C.—Because of the adjusted specific gravity of these emulsions no creaming occurred. However, the purpose of this experiment was to investigate the effect of uniform specific gravity on the rate of change in specific interfacial area with time. As previously found, the relationship of initial specific interfacial area to homogenization pressure was approximately linear although the magnitude of the individual values for specific interfacial area were much lower than those obtained with the corresponding control emulsions (1). The large decrease in specific interfacial area obtained in emulsions of uniform specific gravity was due to the increased frequency of large oil globules. In both the peanut oil and glyceryl ricinoleate emulsions globules up to 10 microns in diameter were observed after 30 days' storage. As in the control emulsions (1) free oil was observed in all of the glyceryl ricinoleate systems. However, deterioration, as evidenced by free oil, was even more rapid, occurring after seven days' storage even in the emulsion homogenized at 3,000 lb. pressure. These results were in accord with previous findings (1) which showed that emulsions having globules over 7–8μ in diameter were unstable and allowed free oil to separate. The more rapid deterioration obtained in this section could thus be correlated with the increased frequency of relatively large oil globules.

Similar results were obtained with peanut oil emulsion. After 15 days' storage, increased frequency of large oil globules caused a sharp decrease in specific interfacial area. In subsequent readings

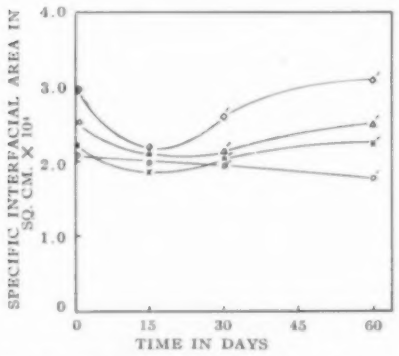

Fig. 3.—Variation in specific interfacial area of peanut oil-brominated oil emulsions stabilized with Tween® 80 and Arlacel® C and stored at room temperature. ′—Separation of free oil. Homogenization pressures, pounds per square inch; O—1500, ×—2000, △ 2500, ◇—3000.

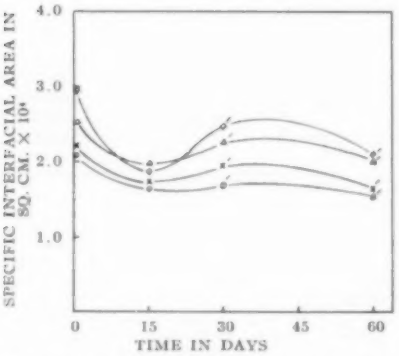

Fig. 4.—Variation in specific interfacial area of peanut oil-brominated oil emulsions stabilized with Tween® 80 and Arlacel® C and stored at 45° C. ′—Separation of free oil. Homogenization pressures, pounds per square inch; O—1500, ×—2000, △—2500, ◇—3000.

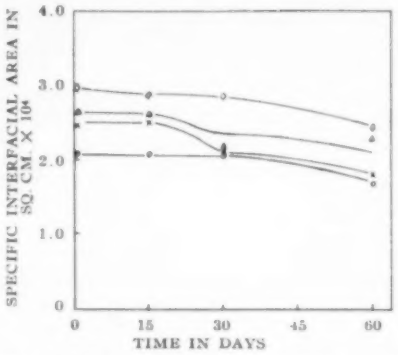

Fig. 5.—Variation in specific interfacial area of glyceryl trioleate-brominated oil emulsions stabilized with acacia and stored at room temperature. Homogenization pressures, pounds per square inch; O—1500, ×—2000, △—2500, ◇—3000.

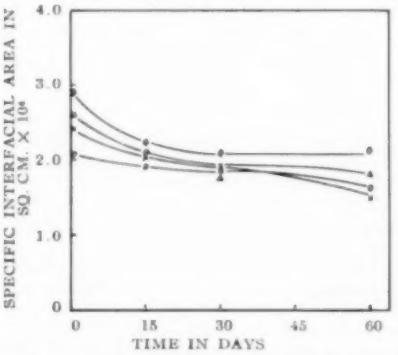

Fig. 6.—Variation in specific interfacial area of glyceryl trioleate-brominated oil emulsions stabilized with acacia and stored at 45° C. Homogenization pressures, pounds per square inch; O—1500, ×—2000, △—2500, ◇—3000.

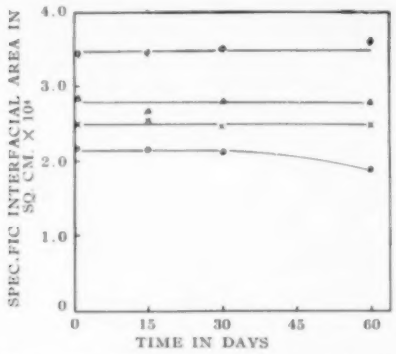

Fig. 7.—Variation in specific interfacial area of cod liver oil-brominated oil emulsions stabilized with acacia and stored at room temperature. Homogenization pressures, pounds per square inch; O—1500, ×—2000, △—2500, ◇—3000.

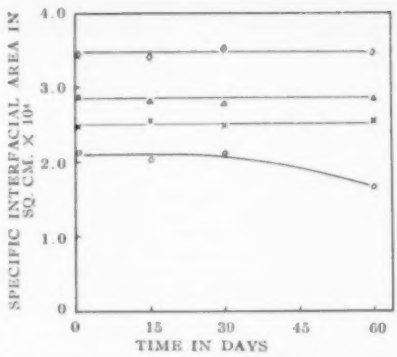

Fig. 8.—Variation in specific interfacial area of cod liver oil-brominated oil emulsions stabilized with acacia and stored at 45° C. Homogenization pressures, pounds per square inch; O—1500, ×—2000, △—2500, ◇—3000.

the frequency of occurrence of the larger oil globules decreased and free oil appeared. Because of the higher relative stability of the smaller oil globules, specific interfacial area appeared to remain unchanged or increased with the separation of free oil. Results of this type are illustrated in Fig. 3 and 4.

Emulsions of Uniform Specific Gravity Stabilized with Acacia.—Glyceryl trioleate and cod liver oil were selected for study. These emulsions were similar in some respects to those discussed in the previous section. The variation of specific interfacial area with homogenization pressure was approximately linear. In addition, the magnitude of interfacial area was less than that obtained with the control emulsions although the differences were smaller than those obtained with emulsions described above.

Results illustrated in Fig. 5 through 8 indicated that the stability of these emulsions was superior to that of the corresponding control emulsions. Whether this increased stability was due entirely to the equalization of specific gravity could not be determined. Possibly the nature of the brominated oil may have contributed to the increased stability although interfacial tension values were not significantly altered. As observed in the control emulsions, deterioration was more rapid in the emulsions stored at 45° than in the emulsions stored at room temperature.

SUMMARY

1. The stability characteristics of some emulsions containing wax in the internal phase were studied. In addition, emulsions of uniform specific gravity were investigated. Stability studies were carried out by means of a size frequency method of analysis.

2. The addition of beeswax to the internal phase of the emulsions studied did not influence stability. Emulsions of this type required the use of heat for emulsification. It was found that the reduced particle size obtained was due only to the higher temperature of emulsification used and not to the wax present in the internal phase.

3. In emulsions where both internal and external phases were of uniform specific gravity and stabilized with Tween® 80 and Arlacel® C deterioration was rapid and characterized by rapid separation of free oil. Deterioration was more rapid than in the control emulsions due to the increased frequency of relatively large oil globules.

4. Deterioration in emulsions of uniform specific gravity stabilized with acacia was relatively slow. Many of the emulsions showed no significant change in specific interfacial area after 60 days' storage.

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The "Roche" Friabilator*

By E. G. E. SHAFER, E. G. WOLLISH, and CARL E. ENGEL

Breakage of medicinal tablets is a source of complaint and is an economic waste. It is desirable, therefore, to formulate and produce tablets which will survive handling in production and in distribution. Estimation of the ability of tablets to survive such handling has been difficult because of the lack of an adequate test to measure the property. A new apparatus which measures resistance to abrasion and shock has been designed. A weighed sample of tablets is subjected in the apparatus to a tumbling action and to the shock resulting from a free-fall of six inches. The loss of weight suffered is a measure of the friability of the tablets. The test has been found useful for the control of plain, compressed tablets, and especially for evaluating new formulations.

A WELL-FORMULATED pharmaceutical tablet should disintegrate within certain time limits depending upon its intended use. At the same time it must be sufficiently "hard" or "tough" to withstand the wear and tear of packaging and of handling in distribution. Several devices for testing the "hardness" of tablets have been described. One of these is the Monsanto hardness tester (1), another is the Strong-Cobb hardness tester (2). Penetration tests, similar to those used for testing the hardness of metals, have been carried out with Vickers diamond hardness testing machine, Shor's Scleroscope, the Sclerograph, and the Rockwell hardness tester (3). In general, these devices measure resistance to compression which, as a rule, is not a serious factor in the life of most tablets. Abrasion and shock are the forces which most often cause tablets to chip or break.

In order to determine the resistance of tablets to wear and tear, Nutter Smith (4, 5) devised a shaking test in which twenty tablets were placed in a closed glass jar and rocked for 15 minutes in a mechanical shaker. Their loss of weight was the measure of their durability. A similar device was employed by Burlinson and Pickering (6), who coined the term "Friability Value" as a measure of resistance of tablets to abrasion.

Spengler and Kaelin (7) carried out an extensive investigation into the relationship between texture, size and shape of tablet and their resistance toward mechanical destruction, abrasion due to rolling, shaking, shock, grinding action, pressure between parallel plates, bending, and indenting by a steel cone. In each of these tests the loss of weight was used as the measure of comparison. In our laboratories we have tried various ideas, including shaking a number of tablets on a 20-mesh sieve in a sieve shaking machine, and using the loss of weight as an indicator of durability.

The fact that such a variety of devices has been used indicates that none was entirely satisfactory. An attempt was made, therefore, to construct a simple apparatus which would not only simulate the abrasion that results from tablets rubbing against one another, against the walls of their containers, etc., but also would subject the tablets to shock such as might be encountered during various steps in packaging, handling, and transport. The "Roche" Tablet Friabilator described in this article was the result. It accomplishes, to a great degree, the objectives sought. A sample of tablets is subjected to abrasion during a four-minute tumbling action and, at the same time, suffers repeated shocks resulting from free-falls of six inches onto a hard surface. The apparatus is constructed of transparent plastic, permitting observation of the tablets during the entire test.

Details of the apparatus are shown by Fig. 1, 2, and 3. The Lucite drum is twelve inches in diameter and $1^1/_2$ inches deep. It is mounted on a shaft which is supported by ball bearings. The

Figure 1.

* Received August 30, 1955, from the Products Control Department, Hoffmann-La Roche, Inc., Nutley, N. J.

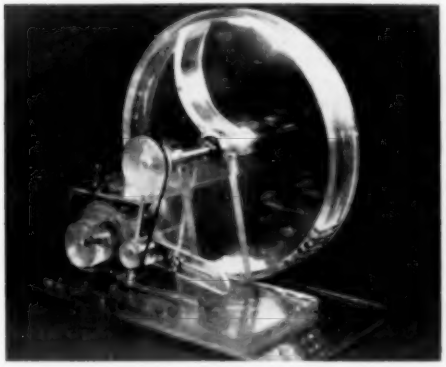

Figure 2.

TEST PROCEDURE

At least 20[1] of the tablets to be tested are brushed free from adhering dust using a soft camel's hair brush. They are accurately weighed and placed in the drum. The front plate is applied to the drum and it is rotated for a timed period of four minutes (100 rotations). If any tablets break or cap during the run, the test is repeated using a new sample. At the end of the rotation period the tablets are removed from the drum, carefully brushed free from adhering dust, and weighed. The loss of weight which has occurred is a measure of the friability of the tablets. The loss may be expressed as milligrams per number of tablets or as per cent of the initial weight of the sample. Repeated capping or breaking is also considered evidence of unsatisfactory friability.

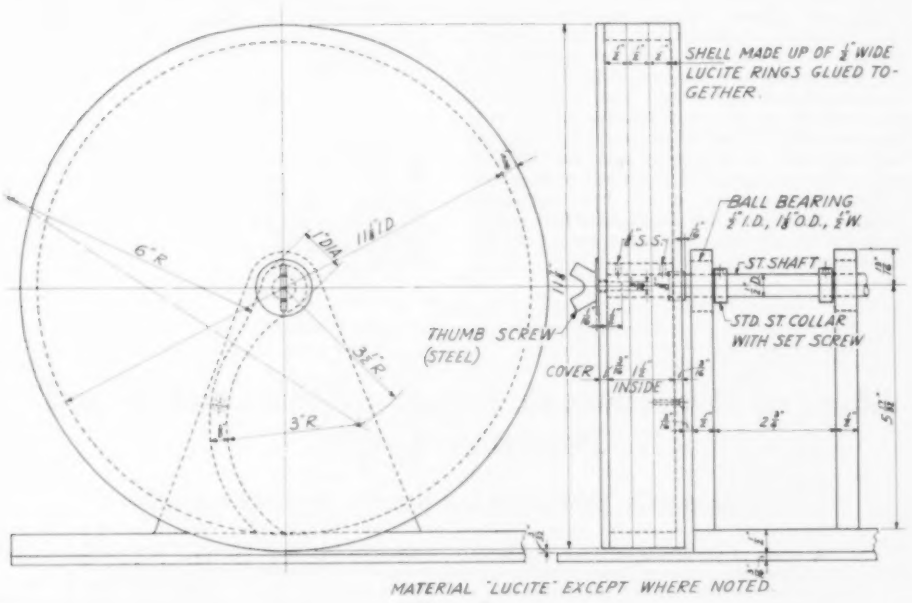

Figure 3.

front cover plate of the drum is removable by unscrewing the single center wing nut. This makes loading and unloading of the apparatus quick and easy. A curved scoop, of special design (Fig. 3), is fixed in the drum and balanced by counterweights inserted in the opposite side wall. The shape and location of the scoop is critical for obtaining agreement between individual Friabilators. The drum is driven via a canvas belt by a Flexo Action Motor at 25 r. p. m. using plastic pulleys and a plastic idler to maintain tension on the belt.

DISCUSSION

Calculating the loss of weight suffered in the test to per cent of initial weight of the sample provides data in easily comparable form. Experience gained in the use of the apparatus over a period of about four years has indicated that plain, cylindrical tablets which lose more than one per cent of their initial weight in the test do not stand up well, as a

[1] The number of tablets is varied depending upon size. A minimum of 20 is used when the average weight is 300 mg. or more. Correspondingly more tablets are used when they have a lesser average weight. A minimum total weight of about 6 Gm. is a useful guide. Maximum total weight appears to depend upon a number of factors, including shape, thickness, etc.

rule, in commercial handling. It must be realized, however, that the behavior of tablets varies with composition, particle size of the granulation, compression pressure, and other factors. It is necessary, therefore, to develop data on each individual formulation in order to set realistic limits of acceptability for the respective products.

The 1% loss limit which we use as a general acceptance limit is based on experience with a variety of tablet formulations. Nevertheless, it was found advisable to use a safety factor in judging new tablets. For such cases, a maximum loss limit of 0.8% of initial weight is permitted until sufficient observations on commercial packages have been accumulated to justify this limit or indicate a more suitable one.

The advisability of using the precautionary safety range for new tablets was indicated by results obtained in experiments designed to evaluate the significance of the figures obtained in the tests. These experiments consisted of packaging tablets of several types in commercial containers and in bulk containers and shipping them by rail across-country (also including transportation by local delivery trucks). When results of Friabilator tests on samples of the tablets were compared with their behavior in the experimental transportation, it was found that, as a rule, tablets which suffered weight loss of less than 0.8% of their initial weight had stood up very well, while those showing more than 1% loss had suffered an undesirable amount of breakage, chipping, or powdering, or all three undesirable effects. The experimental results have been largely confirmed by experience with many lots of a variety of tablets in the trade.

RESULTS

Examples of the results obtained with a number of types of compressed tablets are presented in Table I. Ten successive batches of four different types are listed. The reproducibility of results is considered satisfactory for practical purposes.

TABLE I.—PER CENT LOSS IN WEIGHT ON SUCCESSIVE BATCHES OF TABLETS

Batch	130 mg.	250 mg.	400 mg.	650 mg.
1	0.8	0.6	0.3	0.6
2	0.4	0.4	0.3	0.6
3	0.5	0.4	0.4	0.6
4	0.4	0.3	0.4	0.6
5	0.5	0.6	0.3	0.5
6	0.4	0.5	0.3	0.6
7	0.2	0.5	0.4	0.6
8	0.3	0.5	0.4	0.6
9	0.4	0.5	0.3	0.7
10	0.3	0.4	0.3	0.7

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Effect of Hyperthermic Shock on "Free and Bound" Potassium Levels*

By CHARLES A. LEONARD and JOSEPH W. E. HARRISSON

A study of the levels of "free and bound" potassium in whole blood, plasma and erythrocytes obtained from mice in hyperthermic shock was made using an ultrafiltration method for the separation of "free and bound" fractions. Potassium was determined using a flame photometer. A statistically significant increase in the percentage of "free" potassium was observed in whole blood and erythrocytes but not in the plasma. Total potassium content was increased in the plasma. This is discussed in relation to a current theory of shock.

VARIOUS THEORIES have been proposed concerning the primary cause of the hyperthermic shock syndrome. Today, the opinion seems to be divided among at least three schools of thought. One is the theory of toxemia which proposes that shock may be due to a systemic intoxication by endogenous metabolites formed or liberated from injured tissues (1). These metabolites, often termed "H-substances," possibly include histamine, acetylcholine, potassium, heparin and the several adenine nucleotides such as adenosine triphosphate (2, 3). The theory of local fluid loss proposes that the major factor involved in the production of shock is the loss of circulating fluid into the damaged areas

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(4–6). According to a third theory intestinal bacteria or their products enter the circulation during shock and thus account for at least part of the irreversible shock pattern (7).

The theory of toxemia has received widespread interest as evidenced by the many papers dealing with determinations of the several "shock toxins" during various types of shock. Selye (8) is of the opinion that lack of appropriate techniques in demonstrating these toxins in the blood is responsible for the lack of proof. Furthermore, he has theorized that during shock there is not only an excess production of the toxic metabolites but also an inability to detoxify them.

Disturbances of potassium metabolism are frequently encountered in shock whether traumatic, surgical or associated with other etiologic circumstances. In shock a hypokalemia may be present from an obligatory expansion of extracellular fluids due to circulatory changes (9). On the other hand, oliguria or anuria are not infrequent accompaniments of shock and in such patients the possibility of hyperkalemia must be considered.

The concept that potassium may be a shock-producing metabolite (10, 11), is based on the observations of Kendall and Ingle (12) and Selye (13) that in most conditions which produce the alarm reaction there is a rise in the concentration of serum potassium. However, in some conditions, it is possible to raise the serum potassium to certain levels without producing the alarm reaction. Furthermore, it has been shown that animals with hypokalemia due to overdosage of desoxycorticosterone acetate are especially prone to develop shock (13). From other studies in various forms of shock, several authors have concluded that while a hyperkalemia ultimately occurs, it is a terminal event related to certain tissue changes that precede death rather than the cause of the shock itself (14–17).

With the above-mentioned statement of Selye in mind as well as the current views on the subject as a result of the literature search, it was theorized in this laboratory that perhaps toxic levels of the total "shock-toxins" do not exist in the blood as such but rather as an alteration in the amount which is bound to protein and thus "pharmacologically inactive" and the amount which is not protein-bound and thus "pharmacologically active."

Although the literature contains reports suggesting the existence of bound fractions of some of these "shock-toxins," for example, histamine (18) and potassium (19), there is a paucity of data with this approach to the shock problem in mind. The study reported herein was therefore undertaken to determine whether alterations in protein-bound blood potassium occur in the shock state.

MATERIALS AND METHODS

Animals.—The animals used in these experiments were CFW male mice. They were maintained in air-conditioned quarters in suspended cages with free access to water and Rockland rat checkers at all times. Selection of healthy animals was made in weight ranges averaging twenty-five grams.

Production of Hyperthermic Shock.—The method and apparatus for the production of hyperthermic shock was essentially the same as described by Prinzmetal, *et al.* (20). In brief, the apparatus consisted of a large water bath in which the water was mixed by a stirring motor. The temperature of the bath was regulated by a thermostat so that it varied by no more than ± 0.5° at 60°. The mice to be exposed were rapidly anesthetized with ether. The loose skin on the occipital region was grasped with forceps and the tail held in a similar manner. The animal was then plunged into the scalding water in such a manner that only the head and neck remained above water. The duration of this exposure was ten seconds with an accuracy of ± 0.5 seconds. The animal was then withdrawn and rolled in sawdust to permit partial drying and recovery from anesthesia. Blood was collected one hour after exposure.

Collection of Blood.—The mice were anesthetized with 75 mg./Kg. of sodium pentobarbital administered intraperitoneally in saline. The loose skin over the thoracic cavity was cut away and cardiac puncture made through the intact chest wall using a $^3/_8$-in. 25-gauge needle fitted on a tuberculin syringe. The syringe prior to use was flushed with a solution of heparin sodium in physiological saline containing 1 mg./ml.

Usually about 1 ml. of blood could be withdrawn from each mouse in this manner, although in the case of the animals in deep shock, only about 0.05–0.5 ml. could be obtained. Since at least 7 ml. of blood was required for complete determinations, it was necessary to pool the blood of at least seven normal animals and approximately 25 animals in the shocked groups to obtain this quantity. From each group ten such pools were collected to provide sufficient data for statistical analysis.

Ultrafiltration Apparatus.—This was constructed in the laboratory and is essentially based on that suggested by Stone and Shapiro (21). A single tube from this apparatus is shown in Fig. 1. In use these were connected to a manifold capable of handling thirty tubes at one time.

The ultrafiltration tubes devised for this study were prepared in the following manner. Visking cellulose sausage tubing (8/32 in. OD) was cut into strips about 30 cm. long. One end of a strip was passed through a No. 2 one-hole rubber stopper. A tight seal was then made at this point by inserting a short piece of glass tubing (about 5 cm. in length, 6 mm. OD) inside the cellulose tubing so that the cellulose tubing was pressed firmly against the bore of the rubber stopper. Before use this assembly was soaked in three changes of redistilled water for a total period of about one hour.

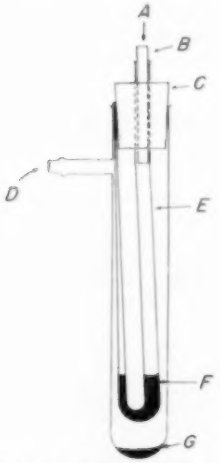

Fig. 1.—Tube from ultrafiltration apparatus. Side arm test tube, 15.0 × 1.9 cm. Kimble resistant glass (A. H. Thomas No. 9460): *A*, air pressure—18–20 p.s.i.; *B*, glass tubing 5 cm. long, 6 mm. OD.; *C*, No. 2—1-hole rubber stopper; *D*, may be covered with Bunsen valve if desired; *E*, Visking cellulose casing 30 cm. long, $^{8}/_{32}$ in.; *F*, sample being filtered; *G*, ultrafiltrate.

Final assembly was accomplished by removing the cellulose tubing from the soak by means of forceps or crucible tongs and shaking off the excess water. The remainder of the water was blown out using filtered compressed air. Care was taken to handle this assembly by the rubber stopper or glass tube only in order to avoid contamination of the cellulose tubing by the fingers. The free end of the cellulose tubing was then folded over for a distance of about 2 cm. and placed next to the rubber stopper so that the remainder of the cellulose tubing was in a shape resembling a hair pin and protruding several centimeters from the stopper. This assembly was placed inside a siliconed side-arm test tube and the rubber stopper pressed firmly in place with the folded-over portion of the cellulose tubing between it and the glass. This procedure eliminated tying knots in the tubing with the attendant danger of stretching or otherwise altering the tubing at that point and inviting leakage or bursting under pressure.

The assembly was inflated by air pressure applied through the glass tubing and tested for leakage before placing a sample within it. A relatively simple way to test for leakage was to fit a Bunsen valve over the side arm, place the complete assembly in a water bath and look for the presence of a steady stream of bubbles either from around the stopper or through the Bunsen valve. This procedure could also be used for immersion in a constant temperature bath if so desired.

The cellulose tubing used was of the same type which finds widespread use in dialysis as well as ultrafiltration processes and under normal conditions is an effective barrier to proteins but permits the passage of electrolytes. Because of the small samples of ultrafiltrate obtained in the course of this study, it was not possible to test the samples for the possible presence of protein. However, in preliminary studies where larger volumes of ultrafiltrate were obtainable, periodic tests using the Biuret test were negative.

The separation of "free" or, more exactly, diffusible potassium from the protein-bound fraction was accomplished by ultrafiltration of the whole blood, plasma, or erythrocytes through the cellulose casing at 18 p. s. i. for twelve hours at room temperature and subsequent analysis of the ultrafiltrate in proper dilution to bring it within range of the flame photometer standards.

Preparation of Samples.—*Whole Blood.*—The tube containing the whole blood was mixed by inverting it three times and samples were taken from this as follows: (*a*) *Hematocrit.*—Whole blood was taken in Van Allen hematocrit tubes which were centrifuged at 3000 r. p. m. for 30 minutes using an International centrifuge, Type 1, SB. (*b*) *Hemolysis Determination Sample.*—A 0.1 ml. sample was taken in a *t.c.* pipet and placed in the buffer solution (see Determination of Per Cent Hemolysis). (*c*) *Whole Blood Total Potassium Sample.*—Two dilutions of 1:100 and 1:200 were prepared by placing 0.1 ml. of whole blood from *t.c.* pipets in redistilled water and making up to a final volume of 10 ml. and 20 ml. (*d*) *Ultrafiltration Sample.*—About 1.5 ml. of whole blood was placed in an ultrafiltration tube using a 2-ml. syringe fitted with a $2^{1}/_{2}$ in. 18-gauge needle tipped with a piece of polyethylene tubing. This procedure minimized the possibility of puncturing the cellulose tubing. The quantity of blood used provided sufficient ultrafiltrate for potassium analysis.

After the above samples were obtained, the remaining blood was poured into 10-ml. Lusteroid centrifuge tubes and spun at 3,000 r. p. m. for 15 minutes. The supernatant plasma was carefully aspirated and placed in a clean tube.

The buffy coat or leukocyte layer was removed from the packed erythrocytes by the method of Randolph and Ryan (22). This method consists of squeezing the Lusteroid tube with a clamp such as a hemostat at a point about 2 mm. below the buffy coat and flushing the mass away with a small portion of saline or plasma. The method effectively removed over 95% of the leukocytes.

Plasma.—Samples of the plasma were prepared as follows: (*a*) *Hemolysis Determination Sample.*—Prepared in the manner described for whole blood. (*b*) *Plasma Total Potassium.*—Two dilutions of 1:20 and 1:50 were prepared by placing 0.1 ml. of plasma from *t.c.* pipets in redistilled water and making to a final volume of 2 ml. and 5 ml. (*c*) *Ultrafiltration Sample.*—About 1 ml. of plasma was placed in an ultrafiltration tube in the manner described for whole blood.

Erythrocytes.—Samples of the packed leukocyte-free erythrocytes were prepared as follows: (*a*) *Hematocrit.*—Collected and prepared in the same manner as for whole blood. (*b*) *Erythrocyte Total Potassium Sample.*—Two dilutions of 1:200 and 1:500 were prepared by placing 0.1 ml. of packed erythrocytes in redistilled water and making to a final volume of 20 ml. and 50 ml. (*c*) *Ultrafiltration Sample.*—Equal volumes of packed erythrocytes and redistilled water, usually about 1 ml. of each, were mixed and placed in the ultrafiltration tubes in the manner described for whole blood. This procedure

was necessary in order to obtain sufficient ultrafiltrate for potassium analyses. Suitable correction for this dilution was made in subsequent calculations.

Preparation of Ultrafiltrates for Analysis.—The ultrafiltrates obtained were generally less than 1 ml. in volume. Since quantities less than 1 ml. were extremely difficult to handle satisfactorily in the flame photometer, dilution of the ultrafiltrate was required to bring it up to 2 ml. or more.

Dilution in the manner mentioned above served a two-fold purpose in that it also enabled the potassium concentration of the samples to be brought within the range of the flame photometer standards. Whole blood and plasma ultrafiltrates were diluted 1:10, erythrocyte ultrafiltrates were diluted 1:100.

Determination of Potassium.—All potassium determinations were made using the Beckman DU spectrophotometer with flame attachment #9200 and oxyacetylene burner. Solutions containing whole blood, plasma, or erythrocytes were prepared for analysis in two different dilutions. When a sufficient quantity of ultrafiltrate was available, these solutions were prepared and analyzed in duplicate. The values reported in the tables of data represent the mean of the values thus obtained. A bracketing procedure was used in which the unknowns were compared with a higher and lower standard in the same concentration range.

Determination of Per Cent Hemolysis in Blood Samples.—It is evident that in handling blood samples in work of this nature some of the erythrocytes will usually hemolyze during the drawing of blood and initial centrifugation in spite of great care. Thus, correction for the potassium contributed to the plasma by these cells was made in order to determine whether or not changes in plasma potassium actually occurred during shock or were merely due to hemolysis *in vitro*. The possibility does exist, however, that in certain forms of shock, hemolysis may occur *in vivo* (23).

TABLE I.—COMPOSITE TABLE OF RESULTS

	Control	Exposure
Total number of mice used	73	232
Mean whole blood hematocrit	41%	56%
Per cent hemolysis		
Range	0.74 – 2.48	1.12 – 2.29
Mean ± S. D.	1.16 ± 0.47	1.50 ± 0.35

The amount of this hemolysis was determined spectrophotometrically according to a method described by Parpart (24) and Chase, *et al.* (25). In brief, the method consists of comparing the extinction at 540 mμ of a sample of whole, hemolyzed blood (diluted 1:100 in a sodium chloride-phosphate buffer mixture) with the extinction of a sample of plasma in the same dilution and solution.

Calculations.—The details of the corrections and calculations for the amount of potassium contributed to the plasma by hemolysis have been given by Hunter (26).

Statistical Methods.—The test of significance between means for small samples employed in this study was the "t" test as discussed in Fisher's text on statistical methods (27). A "P" value less than 0.05 was considered significant.

DISCUSSION

One of the outstanding manifestations of burn shock is hemoconcentration due to extravasation of fluid into the damaged areas. This was demonstrated in the study by increased hematocrit values (Table I) and consequently increased whole blood total potassium levels in the exposure groups (Table II). Although the higher total values for potassium in whole blood can be almost entirely accounted for by the increased hematocrit, the percentage of the potassium which is diffusible through the ultrafiltration membrane is significantly higher than that observed in the controls (Table II). The values for total erythrocyte potassium differ slightly, yet a significantly higher value for percentage diffusible was obtained for the exposure groups. In view of these observations, the percentage of diffusible potassium in the plasma might be expected to be higher as well. However, this was not observed although the plasma total potassium level was significantly higher in the exposure groups even when corrected for the potassium contributed to the plasma by hemolysis.

The findings in this study are in line with the observations of McLean, *et al.* (28), who made similar studies of scalding shock in pigs and dogs. Their studies did not include determinations of diffusible potassium. These authors studied potassium levels in pigs because their skin structure resembles that of man, and, as in man, their erythrocytes contain more potassium than sodium. Dogs were included in the study because their erythrocytes contain more

TABLE II.—COMPOSITE TABLE OF RESULTS

		Total Potassium		Ultrafiltrate Potassium		Per Cent Diffusible	
		Control	Exposure	Control	Exposure	Control	Exposure
Whole Blood	Range	42.3 – 53.4	59.3 – 71.1	5.12 – 7.78	10.5 – 31.9	11.8 – 17.1	15.6 – 46.7
	Mean ± S.D.	47.0 ± 2.96	65.4 ± 3.27	6.62 ± 0.89	19.5 ± 7.91	14.1 – 1.71	29.6 ± 11.35[a]
Plasma (Uncorr. for hemolysis)	Range	4.87 – 6.34	6.44 – 10.65	4.43 – 5.34	4.41 – 9.40	70.0 – 98.8	47.5 – 97.0
	Mean ± S.D.	5.43 ± 0.45	8.69 ± 1.22	4.69 ± 0.29	6.82 ± 1.35	87.0 ± 8.81	79.1 ± 13.66
Plasma (Corr. for hemolysis)	Range	3.92 – 5.91	5.66 – 9.23	3.69 – 4.76	3.40 – 8.50	67.7 – 98.8	41.2 – 96.8
	Mean ± S.D.	4.90 ± 0.57	7.74 ± 1.15	4.17 ± 0.30	5.87 ± 1.31	85.6 ± 9.41	76.5 ± 15.30
Erythrocytes	Range	101.8 – 114.0	103.6 – 117.3	71.6 – 99.4	93.0 – 104.8	63.8 – 95.0	84.6 – 100.0
	Mean ± S.D.	109.8 ± 4.03	108.8 ± 4.39	89.5 ± 8.54	99.0 ± 4.13	81.7 ± 9.13	91.1 ± 5.72[b]

[a] P < than 0.01 significant
[b] P < than 0.02 significant
Values for total and ultrafiltrate potassium are expressed in mEq./L.

sodium than potassium and this difference might aid in determining the source of the additional potassium. The increased levels of plasma potassium they observed in both animals could not be entirely accounted for by *in vivo* hemolysis, hemoconcentration, or loss of potassium from the erythrocyte. The potassium content of the dog erythrocyte characteristically increased during exposure in contrast to the loss of potassium which occurred from the pig erythrocyte. On the basis of their observations they suggested an extravascular source of potassium.

The results of this investigation confirm the increased plasma potassium levels reported by many other authors as well. However, the decrease in diffusible plasma potassium suggests an increased binding of this potassium by the plasma proteins or an increase in the plasma proteins. The loss of diffusible plasma potassium may be due partly to urinary excretion. The source of the additional potassium is not clear. From the data obtained in this study, the source appears to be at least partly the erythrocytes because the total potassium values of the latter are not significantly different whereas the percentage of diffusible potassium is significantly increased.

Various reports in the literature have attributed the rise in serum or plasma potassium to a terminal event rather than to the cause of the shock itself (13–16). On the basis of this, as well as observations made during the course of this study, it might be possible that measurements of diffusible potassium in the blood made at intervals from the time of exposure to scalding up to the time of death, as well as organ studies, would indicate when these changes occur and perhaps identify the source of the additional plasma potassium. For technical reasons the samples in this study were obtained only one hour after exposure.

We have referred to only a few of the many articles, however, several reviews (29–31) provide numerous references.

SUMMARY

1. A study of the levels of "free and bound" potassium in whole blood, plasma, and erythrocytes obtained from mice in hyperthermic shock was made using an ultrafiltration method for the separation of "free" and "bound" potassium. A statistically significant increase in the percentage of "free" potassium was observed in whole blood and erythrocytes but not in the plasma. Total potassium content was increased in the plasma.

2. The above data were corrected for hemolysis. More hemolysis was found in the exposed groups than in the control groups.

3. The construction and use of an ultrafiltration apparatus for the separation of "free" and "bound" potassium is described.

4. The results of the investigation are discussed with suggestions for further studies which may indicate the sources and times at which the observed differences have occurred during the various phases of shock.

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The Measurement of Consistency as a Rheological Property of Ointments*

By RUTH N. HAVEMEYER†

A method is described to designate the consistency of an ointment by a graphical plot and a numerical value. The apparatus simulates the pressure applied and the shearing action involved when ointments are spread. It incorporates a temperature control device and an automatic timing mechanism. Statistical analyses indicate a relative lack of difference in the data obtained for any one ointment, regardless of age, but there is a distinct difference in the data from ointment to ointment among the five tested. The apparatus described may facilitate the study of the effects of temperature and changes in formulation upon consistency.

WITHIN THE PAST DECADE pharmaceutical manufacturers have become increasingly aware of the need for qualitative and quantitative control of the products they market. This is evidenced by assays developed for both raw materials and finished pharmaceuticals, sterility tests on injectables (1), and even taste tests on pediatric preparations (2).

However, little has been done to measure quantitatively the so-called qualitative properties of ointments, which are spoken of vaguely as slip, consistency, spreadability, and feel. Until recently the evaluation of these properties has involved the human element and consequently no positive standards, limitations, or values could be placed upon them.

Consistency has been called "that property of a material by which it resists permanent change of shape and is defined by the complete force-flow relation" (3). The factors which determine the consistency of ointments are varied and complex, and include adhesion, cohesion, viscosity, and rheology. Each of these is in itself intricate.

All of these forces are always present as a composite, and it is difficult to determine where one type ends and another begins; they are inseparable and hence are not easily measurable entities. They are the inherent properties of all substances and affect the manner in which a semisolid will flow.

Rheology is the study of flow and the influence of molecular structure upon the flow characteristics of a material. These characteristics of semisolids are important industrially because they determine the qualities of a finished marketable item. The naturally occurring molecular forces can sometimes be altered, as by the addition of surface tension depressants, but the way in which ingredients are treated prior to and during the manufacture of a product will also greatly affect the properties of that finished product.

The manner in which an ointment spreads may be affected by the pressure applied and the degree of shearing present. For this reason, equipment based on the principle of the penetrometer (4) does not give a true measure of consistency or spreadability. Therefore, it was thought that if a simple apparatus could be devised which would deliver a thin film of ointment over a considerable area, such as is obtained when ointments are applied topically, and if the ease with which it was spread could be measured, then the influence of pressure and shearing force could be more conveniently studied. As a result, an important step towards establishing measurements of the physical properties of ointments would have been taken.

EXPERIMENTAL

The following apparatus (Fig. 1) was found to be suitable. A box 5.89 cm. × 4.45 cm. × 3.48 cm. was made of Dupont 3/16 inch plastic. A narrow slit 3.47 cm. × 0.30 cm. was cut in the bottom of this container and a movable lid which fit snugly inside was made for the top. Two nylon cords 40 cm. long

Fig. 1. Apparatus for measuring the consistency of ointments. A—Heating element, 40.5 cm. × 61.0 cm. × 1.6 cm.; B—aluminum plate, 30.5 cm. × 61.0 cm. × 1.3 cm.; C—Albanene paper;[1] D—Anschutz thermometer No. 1; E—ointment container; F—top load weight, 670.8 Gm.; G—phosphor bronze antenna; H, H'—9 lb. nylon cords; I, I'—pulleys, 5-cm. diameter; J—serrafine clamp; K—pulley load weight holder; L, L'—phosphor bronze contact points.

[1] K & E No. 195-L.

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were attached to the box at each side below the center of gravity and the other ends of these cords were fastened to a small basket called the pulley weight holder.

A testing surface was made from an aluminum plate 30.5 cm. × 61 cm. × 1.3 cm. This was placed over a heating element consisting of coiled nichrome wire imbedded in a base of artificial pressed stone, and the temperature was controlled within a 4-degree range by a Variac. A No. 1 Anschutz thermometer was inserted in a hole drilled in one side of the aluminum plate, and the temperature was kept between 29 and 33°, the average normal range of skin temperature (5, 6). Strips of Albanene paper were cut and placed on top of the aluminum plate and were used as the surfaces for the deposition of the ointments.

Two pulley wheels were mounted on a board placed beneath the heating element. Their upper edges were the same height above the aluminum plate as were the cords attached to the plastic container and drawn over the pulleys. The distance between the pulley wheels was equal to the width of the container, 5.89 cm. Thus the ointment container rested on one end of the flat plate and the cords extended from it over the pulleys. The attached pulley weight holder hung freely so that weights placed therein would lower it and pull the ointment container across the surface of the plate.

An electric timing mechanism was used to measure accurately the interim required for the box to move along the plate. Two phosphor bronze contact points were suspended above the testing surface. The first switch (L) was normally open, the second (L′) normally closed. A small antenna was attached to the ointment container in an upright position. As the container moved, its antenna hit the metal strip in the first unit, making contact and closing the circuit. This started the timer. When the antenna touched the second contact the circuit was broken and the timer stopped.

To operate the apparatus, the plastic container was filled by hand with the ointment to be tested. The movable lid was placed on top and gently pressed down until some ointment was extruded from the slit in the bottom of the box. The bottom outer surface was then cleaned with isopropyl alcohol and dried with a cloth, and the container was placed on the testing surface against a glass backstop at one end of the plate. A piece of lead, weighing 670.8 Gm. and cut to the same linear dimensions as the lid, was placed on the lid to ensure a constant downward pressure on the ointment as the container was pulled across the surface. Next, weights were placed in the pulley weight holder. The minimum pulley load weight was taken as that just sufficient to overcome inertia in starting the container of ointment and to continue pulling it the length of the plate without stopping. Thirty seconds after the lead bar was placed on the lid, the pulley weight holder was released manually. This pulled the container across the paper, and a film of ointment was extruded from the aperture in the bottom. As the container moved, it tripped the automatic timing mechanism and then terminated its operation at the end of the run.

The pulley weights were varied, with a resulting variation in the time required for the distance of 35.4 cm. to be traversed. The maximum pulley weight was chosen subjectively by examination of the ointment films. Whenever a film appeared to be incomplete or spotty, that pulley weight was discarded as being too great.

Using the apparatus and techniques described, ointments representing various types of bases were tested. The following were chosen: (*a*) rose water ointment, U. S. P. (emulsion base), (*b*) anhydrous lanolin, U. S. P. (absorption base), (*c*) white petrolatum, U. S. P. (hydrocarbon base), (*d*) Plastibase,[2] hydrophilic (base with a plastic matrix of submicroscopic interstices which retain a large portion of liquid petrolatum and added liquids), (*e*) yellow ointment, U. S. P. (base with incorporated wax), and (*f*) zinc oxide ointment, U. S. P. (base containing insoluble particles of medication).

All ointments, with the exception of Plastibase, were made by fusion, poured into eight-ounce jars, and hand stirred until they congealed at room temperature. During preliminary experimentation it became apparent that the consistency of the ointments was changing with age. Consequently they were stored for one, six, and twenty days at 29–33° to see if these aging changes could always be detected.[3]

It was found that emulsion bases could not be employed because they were too stiff to be forced through the aperture. However, this could be remedied by replacing the bottom section with one having a wider aperture whenever this type of base was to be tested.

DISCUSSION

For the three age samples of each ointment six runs were made at each pulley weight, and the six-time values thus obtained were averaged ($\bar{t}$). The graphic presentations of pulley weight as a function of $\bar{t}$ (Fig. 2, 3, and 4) give a comparison of the ointments tested at one, six, and twenty days, respectively.

When the study was first undertaken, it had been planned to compare all ointments at a specific weight or within a range of weights. One of the

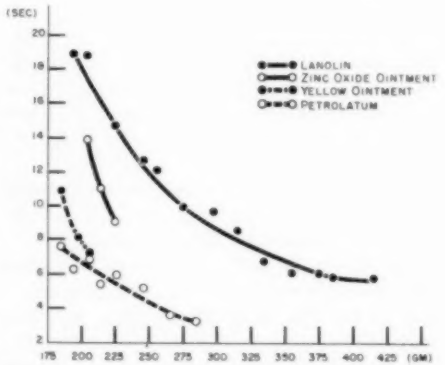

Fig. 2.—$\bar{t}$-Weight curves for all one-day samples.

[2] E. R. Squibb & Sons.

[3] By this time all available Plastibase samples had been stored more than six days. Hence this base was tested at twenty days only.

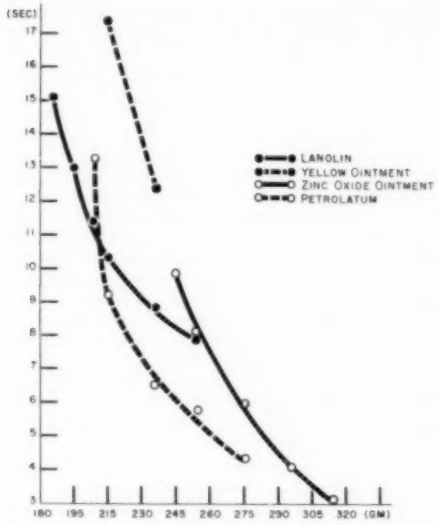

Fig. 3.—*l*-Weight curves for all six-day samples.

ointments would then have been chosen as a standard and set equal to one, and all others numerically compared to it. However, the experimental results showed that this was not feasible, because there was always one ointment, within each age group, which did not fall within the range.

A study of the graphs shows that in Fig. 2 the plot of yellow ointment is not between 205 and 225 Gm., the range in which the other three ointments overlap. In Fig. 3 zinc oxide ointment is the offender and in Fig. 4 it is again yellow ointment. Hence the apparatus and method can be used to best advantage in comparing ointments which have been shown, by preliminary trials, to overlap in a given weight range, or in comparing different batches of the same ointment.

Plots also were made of mean log time as a function of pulley weight, and from these plots the slopes of the lines were determined. This numerical value serves as another means of comparison.

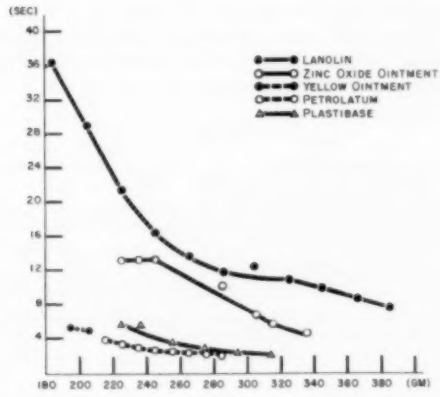

Fig. 4.—*l*-Weight curves for all twenty-day samples.

Of the five ointments tested, lanolin deposited the smoothest looking films on the paper and had a "glassy" appearance. It showed the smallest average slope (-3.497×10^{-3}) in plots of mean log time and pulley weight.

The petrolatum films were also smooth, but not as shiny as those of lanolin.

The appearance of Plastibase was somewhat between that of lanolin and petrolatum. It was found that a Plastibase film left on the paper for an hour or more seemed to soak through and leave a thin layer on the underside. This was not evident with the other bases.

The films of yellow ointment were the most unusual, probably because of the wax particles distributed throughout the petrolatum. It is likely that since these small pieces of wax are much harder than the surrounding petrolatum matrix, they could not be sheared so easily or so readily. Hence each such wax particle on the surface might exert a retarding force. It is possible that as the pulley weight was increased there was insufficient opportunity for these hard pieces of wax to adhere to the paper. The result was a "spot" in the film where the particles passed over. This gave a poor or incomplete film, as judged subjectively, and hence the weight range was very narrow. A microscopic examination of the film was made and showed that the wax particles ranged in size from 35μ to 70μ.

As anticipated, the zinc oxide ointment films were not smooth-surfaced because of the incorporated particles. However, apparently they did not exhibit any drag, for they did not leave streaks nor trails. The particle size, as observed microscopically, was less than 3.57μ. The average slope of the plots of zinc oxide ointment was greater than that for any of the other ointments (-8.819×10^{-3}).

Acceleration curves were made on an electric kymograph at two speeds—1.8 r. p. m. and 3.0 r. p. m. Limiting velocity (V_L) was evidenced when the curve became a straight line. This point varied among the ointments and, in fact, was somewhat different for each ointment at the two speeds. In every case the V_L was not reached until some point beyond the first contact switch. Hence the recorded time is for a period of both acceleration and constant velocity. This could account for the variations in overall time required for the container to travel the 35.4 cm. from the first to the second contact point.

TABLE I.—WEIGHT RANGES AND MEAN TIMES FOR LANOLIN

Weight, Gm.	*l*, Sec.	*t* Range, Sec.
195	17.92	17.35–19.40
205	17.85	16.62–18.65
225	13.79	13.30–14.38
245	11.68	10.80–12.40
255	11.04	10.10–11.62
275	8.87	8.25– 9.55
295	8.75	8.52– 8.90
315	7.46	6.85– 8.15
335	5.93	5.71– 6.22
355	5.17	5.00– 5.35
375	5.15	4.90– 5.30
395	4.98	4.83– 5.20
415	4.99	4.65– 5.30

TABLE II.—WEIGHT RANGES AND MEAN TIMES FOR PETROLATUM

Weight, Gm.	$\bar{t}$, Sec.	t Range, Sec.
185	6.78	6.15–7.30
195	5.40	4.71–6.39
205	5.92	5.20–6.72
215	4.51	4.30–4.89
225	4.86	4.39–5.29
245	4.10	3.80–4.50
265	2.78	2.62–2.90
285	2.22	2.10–2.32

TABLE III.—WEIGHT RANGES AND MEAN TIMES FOR YELLOW OINTMENT

Weight, Gm.	$\bar{t}$, Sec.	t Range, Sec.
185	9.85	8.92–10.30
195	7.07	5.80– 8.42
205	6.27	5.85– 6.68

TABLE IV.—WEIGHT RANGES AND MEAN TIMES FOR ZINC OXIDE OINTMENT

Weight, Gm.	$\bar{t}$, Sec.	t Range, Sec.
205	12.91	12.01–15.69
215	10.08	8.40–11.27
225	8.09	7.30– 9.22

The average time ($\bar{t}$) and the range of time values for six readings taken at each pulley weight are given in Tables I through IV (one day samples). These are presented to show the degree of reproducibility in the time values obtained.

It would appear that the method of manufacture is of particular importance in the case of products such as zinc oxide ointment and yellow ointment, because the degree of aggregation of particles could very well result from the method of their incorporation into the medium. Two rheological factors are yield value and mobility. According to Herdan (7), both of these depend upon the average particle size and weight. As the particle size increases the yield value decreases, but mobility increases. Hence a substance flows more easily when the particles are larger. This is in accordance with the results of microscopic examination of these two films.

Sections of the paper strips (2.0 cm. x 5.0 cm.), coated with the five ointment films, were cut from the beginning, middle, and end of representative runs. These were weighed and compared with control strips of paper (uncoated) to determine the weight of ointment deposited as a film. An analysis of these data indicated that the ointment films were thicker in the middle than at the beginning but that the end sections varied, being sometimes greater and sometimes less than the middle portions. However, these deviations in the weights of the end sections of the ointment films might easily be accounted for by the variation in the paper weight alone. The ointment films were observed to vary from 69.5 mg./10 cm.^{2} to 78.1 mg./10 cm.^{2} The weight of the control paper ranged from 60.1 mg./10 cm.^{2} to 65.4 mg./10 cm.^{2} It was expected that there would be an increase in film thickness as the run progressed, since the container at first accelerated and then attained a limiting velocity.

Several tests were employed for the statistical evaluation of the data, among them the Bartlett chi-square test for homogeneity of variances, and the Cochran test for significance of the largest variance. From them, the following inferences can be drawn: (*a*) as the pulley weight increases, with a concomitant decrease in the time required for the container to travel a standard distance, the amount of variation among the time values decreases, (*b*) an analysis of the variance of $\bar{t}$ indicates there is a distinct difference in the average variance among the ointments, as compared to the relative lack of difference within each ointment, and (*c*) there appears to be a similarity in the slopes of the plots of mean log time as a function of pulley weight for the three age samples of any one ointment, but these coefficients seem to differ for each ointment.

SUMMARY AND CONCLUSIONS

1. An apparatus has been designed and constructed to measure the consistency of ointments. Its more important features are the temperature control in any range above room temperature and the automatic timing mechanism.

2. Five ointments were tested and compared on the basis of the time required for the ointment container to move when being pulled across a surface by various pulley weights.

3. Statistical analyses indicate there is a relative lack of difference in the data obtained for any one ointment, regardless of age, but there is a distinct difference from ointment to ointment among the five tested.

4. Preliminary tests indicate that the spreadability of the ointments studied changes as the ointments age.

5. This apparatus facilitates the study of the effects upon consistency of such variables as temperature and formulation changes. It is believed that additional work would lend further support to the data herein presented.

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A Note on Zein as a Film-Type Coating for Medicinal Tablets

By EDWARD P. WINTERS and DWIGHT L. DEARDORFF

This is a preliminary report on the coating of compressed tablets with a thin film of zein. Previous work in this laboratory using sodium carboxymethylcellulose and hydroxyethylcellulose (1) had indicated that most of the reasons for coating tablets could be satisfied with a very thin film and that use of a thin film offered many advantages over the traditional sugar coating.

Zein is a protein extracted from corn gluten. It is insoluble in water and is soluble in certain organic solvents such as 91% isopropanol (2). It is resistant to microbial attack (3). It is considered to be nontoxic and suitable for use in functional coatings involving the ingestion of relatively small amounts of material (4).

In a typical experiment in the present work the coating fluid was 15% zein, 3% polyoxyethylene sorbitan monolaurate, and 82% of 91% (v/v) isopropanol. Twenty cubic centimeters of coating fluid was added to about 10,000 four-grain (average weight) tablets that were tumbling in a standard 20-inch coating pan. The tablets were allowed to tumble a few seconds until all appeared to be evenly coated. They were then dried with a current of warm air for twenty seconds while tumbling and allowed to rest without tumbling for two minutes before application of the next coat. This procedure was continued using portions of coating fluid ranging from 20 cc. to 10 cc. until a total of 5 coats (90 cc.) had been applied. The total time required was about fifteen minutes. There was no use of prime coat, dusting powder, syrup, extended aging, or oven treatment. The tablets and coating fluid were not warmed before treatment.

The tablets so coated have included aspirin, starch-lactose placebos, phenobarbital, and several types of emblem-bearing commercial uncoated tablets. Several other types of commercial uncoated tablets were so treated, with failure of the coating to adhere to the tablets. The placebos were prepared with a hardness designed for coating. The other tablets were taken from stock and were of such hardness as their makers had thought proper for sale without coating.

Preliminary tests have been carried out comparing tablets so treated with a variety of uncoated and sugar-coated tablets. The tests indicate that this thin film shows promise of giving the following results depending on the characteristics of the original tablet.

When compared to a variety of commercial sugar-coated tablets, the zein-coated tablets seem to exhibit greater resistance to: (*a*) abrasion, (*b*) high humidity (the zein-coated tablets become somewhat less resistant to breakage, but do not show the gross damage that is found with sugar-coated tablets) and (*c*) high temperatures. There was a substantial decrease in disintegration time. When comparing zein-coated tablets with uncoated tablets of the same formulation, the zein-coated tablets seem to show masking of: (*a*) unpleasant odor, and (*b*) unpleasant taste (zein-coated aspirin can usually be held in the mouth for two minutes before the taste of aspirin can be noted); increase in luster (of tablets having little or no luster); greater resistance to abrasion; and a slight increase in disintegration time (typical increases are ten to thirty seconds in distilled water or artificial gastric juice.)

Inspection of the zein-coated tablets shows they retain substantially their original appearance, retain the grooves and emblems initially present, (aiding subdivision and identification), and retain substantially the original tablet weight (1–5 mg. weight gain), weight constancy, shape, and size. The latter factor enhances the ease of swallowing the tablet, compared to the same tablet if made larger by sugar coating.

Consideration of other factors indicates substantial reduction in: coating time and cost, variety of equipment, equipment duplication, space required for equipment, tablet and container size, shipping weight, and storage space, compared to sugar-coated tablets.

Such zein-coated tablets can readily be given an ordinary polishing coat of wax in a few minutes by a standard method. No extended aging is needed before polishing. Application of a film of wax in some instances further increases luster and resistance to high humidity.

Variations in the procedure have involved as many as 12 film coats and have utilized zein in concentrations of 5% to 20% and addition of such materials as polyoxyethylene sorbitan monolaurate in other concentrations (1–10%) or sorbitan sesquioleate (3%). Such variations can also result in desirable tablets. For example, there has been some indication that use of a single film from 50 cc. of a fluid containing 15% zein in 91% isopropanol can give a substantial measure of the desirable attributes described.

It seems probable that many tablet formulations have their own unique coloring problem to be solved. In this work a fluid containing 15% zein, 3% polyoxyethylene sorbitan monolaurate, and 0.03% F. D. C. Red No. 3 dye in 91% isopropanol has given coatings of homogeneous appearance on starch-lactose placebos. The use of colored fluids can also result in a rather unique strongly variegated coating.

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Book Notices

Prufung und Verarbeitung von Arzneidrogen. Erster Band: Chemische Prüfung Allegemeine und spezielle Methoden. By Dr. FRITZ GSTIRNER. Springer-Verlag, Berlin/Göttingen/Heidelberg, 1955. xi + 377 pp. 16 x 24.5 cm. Price DM 36, and Zweiter Band: Verarbeitung Allegemeine und spezielle Methoden. By Dr. FRITZ GSTIRNER. Springer-Verlag, Berlin/Göttingen/Heidelberg, 1955. v + 249 pp. 16 x 24.5 cm. Price 24 DM.

The first volume of this two-volume work is devoted to a presentation of general methods of analyses of botanical drugs and special methods applied to a large number of drugs of vegetable origin. In the section on general methods are included those utilized in the assay of essential oils, the evaluation of bitter principles, the extraction and determination of tannins, and the estimation of the mineral contents of botanical drugs. These chapters are followed by others with discussions on the determination of the swelling qualities of drugs; the extraction of saponins by several methods, the evaluation of mucilaginous drugs, and the determination of water content in drugs. The special part includes methods of evaluation for many of the more widely used botanical drugs and several which in the United States would probably be considered obsolete.

In the preparation of this book, the author has consulted all of the newer literature and has included in his treatise applications of many relatively new chromatographic and instrumental methods.

The second volume is an extension of the first, and like it, is divided into a general and a special part. In the general part, methods of preparation and general methods of evaluation of galenical preparations such as extracts and tinctures are discussed. In the second part, methods of production and evaluation of extractive preparations are discussed.

Both volumes are replete with literature references pertinent and significant to the subjects discussed. The two volumes are provided with separate indexes, but this causes no inconvenience. The printing and binding are of good quality and the books can be recommended to anyone who is concerned with the development of methods of evaluation of botanical drugs and their preparations.

Organic Syntheses. Vol. 35. T. L. CAIRNS, Editor. John Wiley and Sons, Inc., New York, 1955. vi + 122 pp. 15.5 x 23.5 cm. Price $3.75.

Organic Syntheses, an annual publication of methods for the preparation of organic chemicals, now in its thirty-fifth year, is so well known to organic chemists and others who have occasion to need dependable preparative methods that its origin and background require no description. The format and style of the annual volumes change little from one year to the next and a uniform size has been maintained from the beginning. The first twenty-nine volumes have been issued in three collective volumes and this practice undoubtedly will be continued by the publisher. In Volume 35 methods of preparation are given for the following compounds: 1-acetylcyclohexanol; 2-amino-3-nitrotoluene; 3-benzyl-3-methylpentanoic acid; *p*-bromomandelic acid; *sec*-butyl α-*n*-caproylpropionate; *n*-butyl glyoxylate; 2-butyn-1-ol; chloro-*p*-benzoquinine; N-chlorobetainyl chloride; α-(4-chlorophenyl)-γ-phenylacetoacetonitrile; 1-(*p*-chlorophenyl-3-phenyl-2-propanone; 2-chloropyrimidine; Δ^4-cholesten-3,6-dione; Δ^4-cholesten-3-one; cholesterol, Δ^5-cholesten-3-one, and Δ^4-cholesten-3-one; diacetyl-*d*-tartaric anhydride; diethyl mercaptoacetal; diethylthiocarbamyl chloride; 2-(dimethylamino)pyrimidine; ethyl diethoxyacetate; ethyl isocyanide; ethyl N-phenylformimidate; 2-furfuryl mercaptan; guanylthiourea; hexamethylbenzene; *o*-methoxyphenylacetone; 5-methylfurfuryldimethylamine; 4-methyl-6-hydroxypyrimidine; 1-methyl-3-phenylindane; methyl 2-thienyl sulfide; β-methyl-δ-valerolactone; *trans*-*o*-nitro-α-phenylcinnamic acid; 2-phenylcycloheptanone; 1-tetralone; thiobenzophenone; and *p*-toluenesulfenyl chloride.

The book is provided with a cumulative subject index to Volumes 30 to 35, inclusive.

Official Methods of Analysis of the Association of Official Agricultural Chemists. 8th ed. WILLIAM HORWITZ, Editor. Association of Official Agricultural Chemists, Washington, 1955. xvi + 1008 pp. 16 x 26 cm. Price $12. domestic; $12.50 foreign.

New and revised methods of analysis adopted by the Association of Official Agricultural Chemists since the publication of the 7th edition in 1950 are included in this new 8th edition, which has been expanded by approximately 100 pages. The expansion occurs chiefly in the fields of pesticides, flavoring extracts, drugs, extraneous materials, microchemical methods, and nutritional adjuncts, including vitamins. While nearly every section of the book has undergone some degree of revision, those of greatest interest to the readers of this Journal are in the section on drugs.

In this section one finds among the additions a thermal (melting point) method for determining the stereochemical composition of amphetamines, and directions for determining the optical-crystallographic properties of certain drugs. Reference tables of these properties are provided for antihistamines, barbiturates, sulfonamides, sympathomimetic amines, and reineckates of quaternary ammonium compounds. A new section on hormones has been added to the drug chapter, including methods of analysis for physiologically potent hormones heretofore determined mainly by bioassay. Instrumental methods and newer techniques such as chromotography have been utilized in the 8th edition of *Official Methods of Analysis*. Thus a new chapter on spectrographic methods has been

added and in this connection a new and consistent terminology has been adopted. Chromatographic and spectrophotometric methods have been adopted for the isolation and determination of coal-tar colors from food in place of the older dyeing on, and stripping from, wool cloth, followed by indentification of the colors by means of spot reactions.

The chapter formerly entitled "Vitamins" has been replaced by the title "Nutritional Adjuncts," ". . . to convey a recognition of the potential of nutritionally significant factors such as amino acids and antibiotics." No amino acids or antibiotics are covered in this chapter, and it may be hoped that a better title can be devised prior to the publication of the 9th edition, five years hence, for a chapter intended to cover amino acids, antibiotics, and vitamins.

Many of the procedures described in *Methods of Analysis* are of pharmaceutical interest and the book will continue to be used to a great extent by pharmaceutical chemists involved in the application of analytical methods to drugs and chemicals.

Methoden der organischen Chemie. Vol. 3, Part 2, Physikalische methoden. (Houben-Weyl.) Georg Thieme Verlag, Stuttgart, 1955. xxviii + 1078 pp. 18.5 x 26 cm. Price $44.30.

This volume represents the fifth in the new series of *Houben-Weyl*, the publication of which appears to be proceeding according to a planned schedule announced at the time distribution of the earlier volumes began. The appearance of each new member of the series illustrates and emphasizes the great importance of this monumental work to organic chemistry. The application of physical methods to organic chemical research has developed tremendously during the past two decades. The editors of Volume III, part 2 of *Methoden der organischen Chemie* have exercised excellent judgment in devoting this volume to physical methods used in organic chemical research.

The volume is divided into twenty-one chapters, each of which presents a clear exposition of the principles and applications of the specialized subjects covered. The first chapter covers a general treatment of conductivity and is followed by representative chapters on methods of determining hydrogen ion concentration and potentiometric and conductometric titrations. Other chapters cover such subjects as quantitative electrophoresis, polarography as applied to organic compounds, and methods of determining dielectric properties. The electron microscope is adequately described and its application to chemical research discussed. All phases of spectroscopy are discussed in a series of three chapters including spectrophotometric methods in the ultraviolet and infrared ranges as well as an excellent chapter on Raman spectroscopy. Other chapters devoted to older and more conventional methods used in characterizing organic compounds are also adequately covered. Each contributor to this volume is to be congratulated upon the excellence of the chapter for which he has been responsible.

This volume, like those which have preceded it in the series, is documented throughout and the references employed extend through the end of 1954. An author index and a highly functional subject index are provided. The printing and binding are of the excellent quality usually employed by the Georg Thieme Verlag.

The Analysis of Drugs and Chemicals. By NORMAN EVERS and WILFRED SMITH. Charles Griffin and Co., Ltd., London, 1955. xii + 546 pp. 15.5 x 23 cm. Price 60s.

This book originally appeared under the authorship of Norman Evers and G. D. Elsdon in 1929. Quite naturally the present authors found it necessary to revise completely the entire book and for this reason it is not to be considered as a second edition of its precursor. *The Analysis of Drugs and Chemicals* is designed to supplement the British Pharmacopoeia and the British Pharmaceutical Codex. The methods described, however, frequently go quite beyond those of the B. P. and the B. P. C. Methods are also provided for a number of drug products which are not included in either of the British books of official standards, such as chemicals used in food manufacture and in agriculture. The book is definitely not a textbook, but is intended for use by qualified chemists. Three chapters are devoted to brief discussions of physical methods, volumetric methods, and general methods used in the analysis of pharmaceutical products. Other chapters cover inorganic chemicals, organic chemicals, natural compounds, and galenicals and other pharmaceutical preparations. A short chapter on the use of statistics in chemical analysis is provided and the value of the book is enhanced by a series of appendixes providing data of importance in determination of the quality of drug products. The book is provided with a satisfactory index and the printing and binding are of excellent quality. While designed for use by British chemists, *The Analysis of Drugs and Chemicals* would constitute a useful addition to the reference books required by pharmaceutical chemists engaged in analytical procedures.

The Merck Veterinary Manual. Merck & Company, Inc., Rahway, N. J., 1955. ix + 1385 pp. 11.5 x 17.5 cm. Price $7.50.

In general, this new Merck publication resembles in format and style another publication entitled *The Merck Manual of Diagnosis and Therapy*, first published in 1899 and now in its eighth edition, which was published in 1950. With this experience in the compilation and publication of a manual used by the medical and allied professions, it is quite natural that the *Merck Veterinary Manual* has followed the design of the earlier publication.

The Merck Veterinary Manual contains 389 principal chapters on the diagnosis and therapy of diseases in animals, including poultry. Part I, divided into seventeen sections, covers the diagnosis and therapy of diseases of large and small domestic animals. Part II lists poisonous plants of the United States, in tabular form for quick reference, and covers the toxicology, including herbicides, insecticides, and bacterial toxins to which animals may be exposed. Part III is devoted to diseases of poultry and Part IV deals with the health and disease problems of fur, laboratory, and zoo animals. Part V contains data related to public health and a wealth of other useful supplementary information.

Therapeutic reference is made to more than 900 prescriptions arranged according to their thera-

peutic action, at the end of each section, and adapted to the various species. These prescriptions, in general, require official drugs, although in a few instances products are referred to by proprietary names.

In the course of preparation of the Manual, more than 200 authorities in various fields of verterinary medicine contributed either as authors or consultants with the editorial directors of the Merck Verterinary Research Department. The book is provided with an unusually extensive subject index with cross-index entries provided to increase its reference value.

Hydrogen Peroxide, A. C. S. Monograph No. 128. By WALTER C. SCHUMB, CHARLES N. SATTERFIELD, and RALPH L. WENTWORTH. Reinhold Publishing Corporation, New York, 1955. xiii + 759 pp. 16 x 23.5 cm. Price $16.50.

This book represents a worthy addition to the American Chemical Society's series of chemical monographs. It presents, for the first time in English, a comprehensive treatise on the manufacture, properties, technology, and uses of hydrogen peroxide.

The book begins with the history and technical development of hydrogen peroxide, which is followed by descriptions of currently used and potential methods of manufacture. Other chapters are devoted to such topics as purification and handling; physical, thermodynamic, electrical and chemical properties; molecular structure and association; catalysis and reaction mechanisms; stability; methods of qualitative and quantitative analysis; and uses of hydrogen peroxide including bleaching, oxidation, and as a source of energy. The last chapter deals with a preparation of inorganic peroxides and superperoxides and describes the metallic and nonmetallic peroxy compounds, peroxy acids, and salts.

More than 2,500 literature references are cited in this new monograph. Hydrogen peroxide is a versatile chemical having applications in many of the chemical industries. To all who are concerned with the applications of peroxides in one way or another, this book is indispensable. Its value is enhanced by an author index and a thoroughly satisfactory subject index. The printing and binding are of the same quality that has characterized the A. C. S. series of chemical monographs from the beginning.

Organic Insecticides—Their Chemistry and Mode of Action. By ROBERT L. METCALF. Interscience Publishers, Inc., New York, 1955. x + 392 pp. 16 x 23.5 cm. Price $8.50.

This book is an outgrowth of a monograph entitled "The Mode of Action of Organic Insecticides," published in 1948 as Review No. 1 of the Chemical-Biological Coordination Center, National Research Council, Washington, D. C. It is designed to furnish information regarding the chemistry and mode of action of insecticides, the relation of their chemical structure to toxicity, their metabolism in plants and animals, and their toxic hazards to higher animals. Most of the available information on these subjects has been summarized into a coherent pattern dealing with organic insecticides.

The book includes fourteen chapters and covers a number of different types of organic insecticides with chapters on the synergistic action of insecticides and insecticide resistance.

The book should be of particular value to the investigator in the field of insecticides, toxicology, and the development of new insecticides. The book is adequately documented throughout with references to the original literature and it is provided with an excellent subject index.

Classics of Biology. By AUGUST PI SUÑER. Authorized English translation by Charles M. Stern. Philosophical Library, Inc., 1955. x + 323 pp. 14.5 x 22.5 cm. Price $7.50.

This survey, written by an outstanding biologist, covers the high points of progress in the study of biology by providing fragmentary portions of the philosophical theories which have been propounded in different ages up to our own time. Famous controversies are discussed and illustrated by textual extracts from the outstanding publications of more than sixty writers. Through this device the reader is introduced to thinkers whose fame may not be too well known among English speaking peoples.

The book is provided with an excellent index and the book is attractively printed and bound. It probably will appeal most to teachers of biology, philosophy, and the history of science, but it also should be of interest to the general reader, who will find the contents of the book stimulating and well worth reading.

BOOKS RECEIVED

A Short History of Medicine. By ERWIN H. ACKERKNECHT. The Ronald Press, New York, 1956. xviii + 258 pp. 14 x 21 cm. Price $4.50. Illustrated.

Laboratory Manual for Introduction to Pharmacy. HENRY M. BURLAGE, Editor. McGraw-Hill Book Company, Inc., New York, 1956. xv + 271 pp. 21.2 x 28 cm. Paper back. Price $4.50.

The Systematic Identification of Organic Compounds. 4th ed. By RALPH L. SHRINER, REYNOLD C. FUSON, and DAVID Y. CURTIN. John Wiley & Sons, Inc., New York, 1956. ix + 426 pp. 14.5 x 22 cm. Price $6.

Textbook of Pharmacognosy. 3rd ed. By T. E. WALLIS. J. & A. Churchill Ltd., London (Little, Brown and Company, Boston) 1955. xi + 578 pp. 15 x 22.2 cm. Price $7.50.

Methoden der organischen Chemie (Houben-Weyl). By EUGENE MÜLLER. Vol. IX, Schwefel-, Selen-, Tellur-Verbindungen. Georg Thieme Verlag, Stuttgart, 1955. xix + 1337 pp. 19 x 26 cm. Price DM 196.20.

Collected Papers of Dr. I. C. Rubin, 1910–1954. Published privately, 1954. No consecutive pagination. 6.5 x 16 x 23.5 cm.

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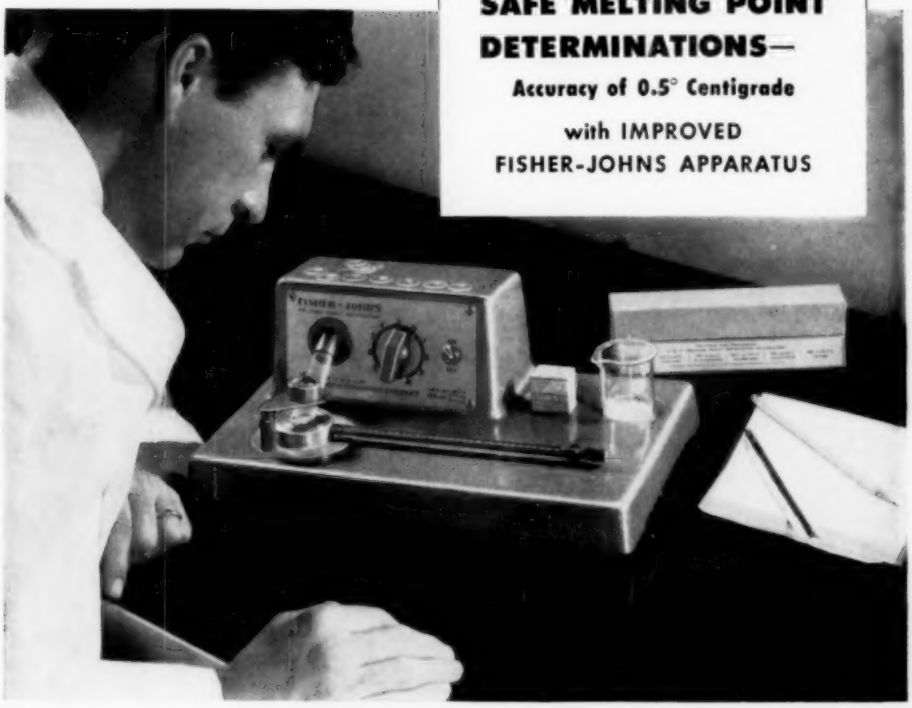

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